ISSN 0704-3716

Canadian Translation of Fisheries and Aquatic Sciences

No. 5132

Studies on the formation of the house in appendicularians (Oikopleura dioica Fol)

W.F. Korner

Original title: Untersuchungen uber die Gehausebildung bei Appendicularien (Oikopleura dioica Fol)

In: Z. Morphol. Oekol. Tiere 41: 1-53, 1952

Original language: German

Available from:

Canada Institute for Scientific and Technical Information
National Research Council
Ottawa, Ontario, Canada K1A 0S2

1985

91 typescript pages

Fisheries & Oceans
LIBRARY
DEC 2 1986
BIBLIOTHÈQUE
Pêches & Océans

Secretary of State / Secrétariat d'État

90-00098/

MULTILINGUAL SERVICES DIVISION – DIVISION DES SERVICES MULTILINGUES

TRANSLATION BUREAU BUREAU DES TRADUCTIONS

LIBRARY IDENTIFICATION – FICHE SIGNALÉTIQUE

CTFAS 5132

Translated from - Traduction de	Into - En
German	English

Author - Auteur

Wilhelm Friedrich Körner

Title in English or French - Titre anglais ou français

Studies on the formation of the house in appendicularians (Oikopleura dioica Fol)

Title in foreign language (Transliterate for[illegible]aracters)
Titre en langue étrangère (Transcrire en ca[illegible]s romains)

Untersuchungen über die Gehäusebildung bei Appendicularien (Oikopleura dioica Fol)

Reference in foreign language (Name of book or publication) in full, transliterate foreign characters.
Référence en langue étrangère (Nom du livre ou publication), au complet, transcrire en caractères romains.

Z. Morph. u. Ökol. Tiere

Reference in English or French - Référence en anglais ou français

Journal of Animal Morphology and Ecology

Publisher - Editeur	DATE OF PUBLICATION / DATE DE PUBLICATION			Page Numbers in original / Numéros des pages dans l'original
Springer-Verlag	Year / Année	Volume	Issue No. / Numéro	1-53
Place of Publication / Lieu de publication				Number of typed pages / Nombre de pages dactylographiées
Heidelberg	1952	41	--	90

Requesting Department / Ministère-Client: DFO

Translation Bureau No. / Notre dossier nº: 1655826

Branch or Division / Direction ou Division: SIPB, Ottawa

Translation (Initials) / Traducteur (Initiales): LM

Person requesting / Demandé par: A.T. Reid

FEB 0 1985

Your Number / Votre dossier nº:

TRANSLATION NOT EDITED FOR PUBLICATION
TRADUCTION NON DESTINEE A LA PUBLICATION

Date of Request / Date de la demande: 03/10/84

Canada

SEC 5-111 (Rev. 82/11)

Secretary of State | Secrétariat d'État

MULTILINGUAL SERVICES DIVISION – DIVISION DES SERVICES MULTILINGUES

TRANSLATION BUREAU | BUREAU DES TRADUCTIONS

Client's No.–N° du client	Department – Ministère	Division/Branch – Division/Direction	City – Ville
	DFO	SIPB	Ottawa
Bureau No.–N° du bureau	**Language – Langue**	**Translator (Initials) – Traducteur (Initiales)**	
1655826	German	LM	FEB 6 1985

Z. Morph. u. Ökol. Tiere, Vol. 41, 1952, pp. 1-53.

From the Norderney Research Station*, the Max Planck-Institute for Marine Biology** in Wilhelmshaven, and the Zoological Department of the Darmstadt Institute of Technology***

Studies on the Formation of the House in Appendicularians (Oikopleura dioica FOL)[1]

Wilhelm Friedrich Körner

With 50 Figures (66 Individual Figures)

(Received on 15 December 1951)

Table of Contents

		Page (in source text)
I.	Introduction	2
II.	Material and Methods	5

Translator's notes:

*Forschungsstelle Norderney

**Max Planck-Institut für Meeresbiologie

***Zoologisches Institut der Technischen Hochschule Darmstadt

[1]Doctoral thesis from the Darmstadt Institute of Technology.

SEC 5-25 (Rev. 82/11)

Canada

TRANSLATION NOT EDITED FOR PUBLICATION
TRADUCTION NON DESTINEE A LA PUBLICATION

III. Description of the Material 6

IV. Structure and Function of the Feeding Apparatus 11

A. Morphology of the Feeding Apparatus 12

B. Function of the Filter System 15

V. The Oikoplastic Epithelium 17

VI. The Intracellular Secretion Processes 20

A. General Secretion Process 20

B. The Individual Phases 21

a) Formation of Prosecretions 21

b) Formation of the Final Secretion 23

c) Secretion of the Secretory Strands 24

VII. The Extracellular Processes during Formation of the House 27

A. The Development of the Final Fine Structure of the House 27

B. The Formation of the Feeding Apparatus 28

a) Secretion Mechanism of the Giant Cells 28

b) Secretion Mechanism of the Filter Producing Cells 29

c) Secretion Mechanism of the Boundary Cells 31

C. The formation of the Incurrent Grids 32

VIII. Chemical Composition of the House 36

IX. Development of the "Exuvial Bodies" 39

A. Mechanism of Cell Discharge 40

B. Problem of Cell Restitution 43

C. Chemical Properties 44

D. Function of the "Exuvial Bodies" 44

E. Question of Cell Constancy 45

F. Definition of "Exuvial Bodies" 46

X. Affinities of the Appendicularians with the other Tunicates Based on the Present Findings 46

A. Formation of House and Tunic in the Tunicates 46

B. Chemical Comparison of the Main Constituents of the Tunic Substance 47

Summary 48

Literature 50

I. Introduction

All Tunicata have a common character, namely they secrete a tunic. This formation of a tunic has been studied so far in some detail only in the ascidians and in salps [SEELIGER 1893 (1), O. HERTWIG 1873, KOWALEWSKY 1892, SEMPER 1975, RIES 1937 (1)]. In the appendicularians, LOHMANN[1] focused his studies on the development and shape of the house, but we know nothing so far about the formation of the house substance.

From the findings available to date, we can assume that the formation of the appendicularian house is homologous to the formation of the tunic in the ascidians and salps [SEELIGER 1893 (2)]. Nevertheless, considerable differences exist between the formation of the tunic in the ascidians and salps on the one hand, and the formation of the house in the appendicularians, on the other hand.

The sessile ascidians and the free-swimming salps form a tunic which they retain throughout their life. The appendicularian house, on the other hand, is formed on the free-swimming animal, and the house is shed at certain intervals, as required, and replaced with a new house (LOHMANN 1933).

> In contrast to the ascidians and salps whose tunic covers the entire body surface [HUUS 1937, NEUMANN 1935 (1)], the house of the appendicularians is restricted to a more or less large area of a simple epithelium on the anterior part of the body, which KLAATSCH (1895) has designated "oikoplastic epithelium".

---

[1] The papers will be mentioned individually in the course of this study.

> Tunic production in the ascidians and salps occurs only once, but is continues until the tunic reaches a certain thickness during the animal's life [HUUS 1937, NEUMANN 1935 (1)]. Tunic production in the appendicularians, on the other hand, seems to be a cuticular exuviation process since several houses are successively produced and secreted within a certain time interval (LOHMANN 1931).

In the representatives of the family Oikopleuridae, the oikoplastic epithelium extends over the entire anterior part of the body; but it does not cover the gonads and the tail base (Fig. 1). The oikoplastic epithelium is a mosaic of polygonal cells of varying sizes (Fig. 2). These cells can produce a well-functioning house only when their interaction is perfectly coordinated (LOHMANN 1933). Particularly conspicuous are two regions of large cells located in this cell complex laterally on either side. These are the FOL's oikoplasts which lie near the gill slits and are responsible for the formation of a highly complex feeding apparatus, and the EISEN's oikoplasts which are located near the tail base and are said to form the grid structure of the incurrent orifices [LOHMANN 1899 (1)].

> BÜCKMANN [1923 (2)] selected individual groups of cells which had a characteristic location and shape within the oikoplastic epithelium and combined them into particular regions. Such a division is helpful for orientation purposes but remains arbitrary as long as we do not know precisely which of the cells produce the individual parts of the house. Only in the

p. 3

case of FOL's and EISEN's oikoplasts are we certain about the final product of their secretory activity. It may well be that the individual groups of cells as represented by BÜCKMANN [1923 (2)] interact in the production of corresponding parts of the house, but the manner in which the individual cells secrete and combine their products is as yet unknown. Notwithstanding this, I have retained this division and have adapted it to p. 4 Oikopleura dioica FOL since it permits easier orientation on the oikoplastic epithelium (Fig. 3).

All tunicates have a simple epithelium which secretes, on its entire surface, a transparent gelatinous cuticle which consists primarily of polysaccharides (BRIEN 1948). While chemical, roentgenographic, and electron-microscopic methods have established that the tunic substance in the ascidians is identical with plant cellulose[1] [LOEWIG AND KÖLLIKER 1846, AMBRONN 1899 (1), F.E. SCHULZE 1863, SAINT-HILAIRE 1931, SUTRA 1932, FREY-WISSLING 1950], the substance of the appendicularian house has so far eluded identification (FOL 1872, LOHMANN 1933, BRIEN 1948). It has been established beyond doubt that the appendicularian house does not consist of cellulose since the microchemical tests commonly used for cellulose by previous investigators have always been negative (FOL 1872, SALENSKY 1903, LOHMANN 1933). My own tests produced the same results. It was therefore necessary to determine the

---

[1] BRIEN (1948) reported recently that the tunic of the ascidians consisted of a chitinous substance which, however, contained much cellulose. The appendicularian house, on the other hand, is said to consist of a purely chitinous mass.

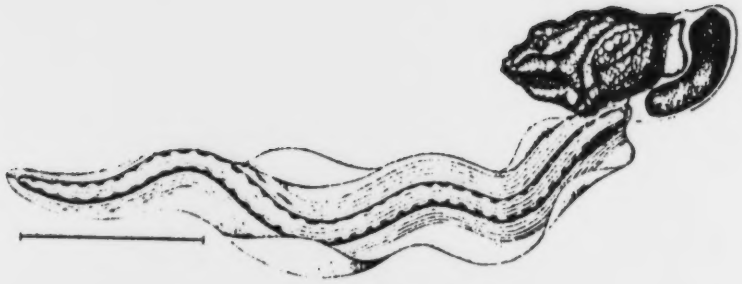

Fig. 1. Oikopleura dioica FOL. Animal without house; drawn from life Scale 500 µ.

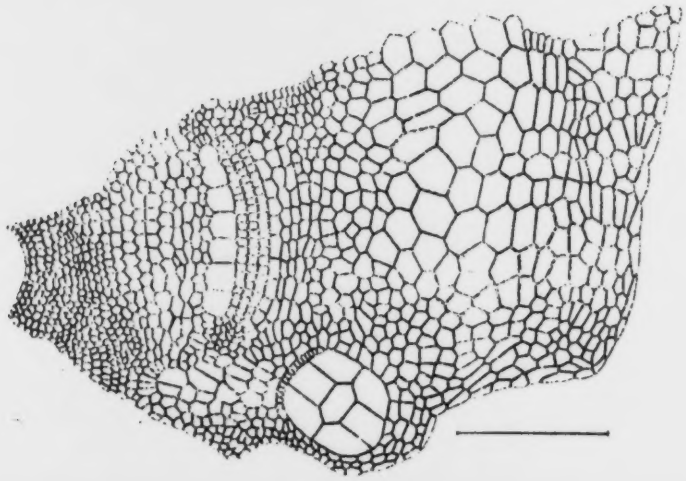

Fig. 2. Oikopleura dioica FOL. Left half of the oikoplastic epithelium which covers the anterior part of the animal's body; after preparation of the epithelium and whole preparations. The cell nuclei have been omitted from the drawing. Scale 150 µ.

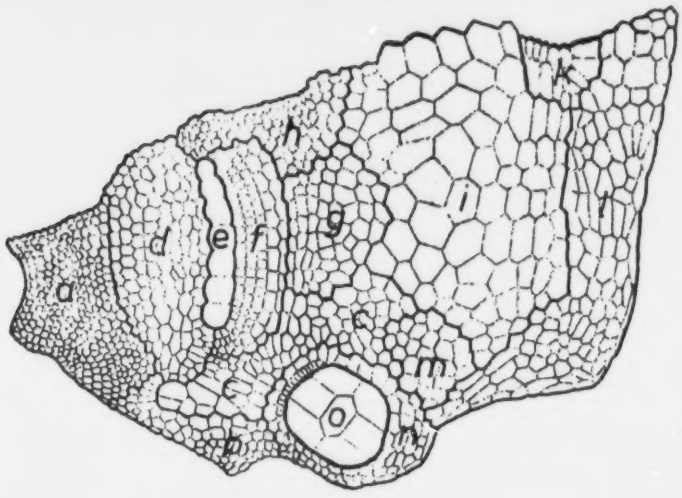

Fig. 3. Division of the oikoplastic epithelium into regions as suggested by BÜCKMANN:
a. oral region;
b. pharyngeal region;
c. oblique line;
d. anterior cells from FOL's oikoplasts;
e. giant cells from FOL's oikoplasts;
f. filter producing cells and boundary cells from FOL's oikoplasts;
g. MARTINI's field;
h. anterior rosette;
i. dorsal cells;
k. posterior rosette;
l. posterior boundary cells;
m. IHLE's field;
n. border around EISEN's oikoplasts;
o. giant cells from EISEN's oikoplasts.

chemical composition of the appendicularian house by means of currently available microchemical methods as a basis for a subsequent study of the secretion processes. Needless to say, detailed biochemical studies are still required since our findings provide no more than an overview.

In the ascidians, the tunic is transformed into a kind of living tissue by the incorporation of connective-tissue cells and vessels [O. HERTWIG 1873, HUUS 1937, RIES 1937 (1)]. The appendicularian house, on the other hand, is solely the result of cell secretion and is regarded as being free of cells according to studies available so far (LOHMANN 1933). The house substance constitutes a cuticle which is composed of fibrils, membranes and a gelatinous mass. It separates from the epithelium during the "moulting process", inflates to form the house and the filter apparatus, and is again shed when it has served its purpose (LOHMANN 1933). p. 5

According to LOHMANN [1899 (1)] who used Oikopleura albicans LEUCKART for his studies, the secreted cuticle consists of a gelatinous mass and is composed of closely spaced fibrils which arise vertically from the epithelium, contain transverse filaments, and are fused into a uniform mass by a binding substance. The cuticle develops in three stages (LOHMANN 1933).

> The species of the subgenus Vexillaria LOHMANN, to which Oikopleura dioica FOL belongs, also secrete so-called "exuvial bodies" beween the development of two houses. These exuvial bodies differ in shape from one species to another. They may be spherical, plate-like, tube- or club-shaped, and their presence is restricted to certain regions on the oikoplastic epithelium (LOHMANN 1933). Their function, according to LOHMANN (1933) is to facilitate the separation of the new house rudiment from the epithelium.
>
> On average, the house is shed every 3-4 hours and replaced with a new house within about one hour. We can therefore assume that the animal builds at least 5-6 houses each day [FOL 1872, SEELIGER 1893 (2)].

No information is available so far about the cellular activity during the formation of the house. We do not yet know which processes occur in the interior of the cells of the oikoplastic epithelium and which functions are carried out by the individual cells. It is equally unclear what mechanical and physico-chemical processes take place outside of the oikoplasts.

The question, therefore, remains as to what kind of "preparations" occur in the organism which eventually result in the development of a highly complex and perfectly functioning house. The present investigations are aimed at clarifying these matters.

I am greatly indebted to my esteemed mentor, Prof. Dr. W. E. ANKEL, for encouraging me to undertake this study and for his continuous and generous support throughout the present work.

II. Material and Methods

Specimens of the species Oikopleura dioica FOL collected in the Deutsche Bucht (= German Bight) were used as study material, but these specimens differ in two characters from the species Oikopleura dioica FOL described by FOL (1872) and LOHMANN (1896). For this reason, I consider it appropriate to include a brief description of the animal in the present study (see Chapter III).

Earlier studies on the formation of the house were largely carried out on Oikopleura albicans LEUCKART [LOHMANN 1899 (2)]. Since I was unable to obtain specimens of that species, I used the smaller species Oikopleura dioica FOL for my studies. The main difficulties involved in such studies are the supply of sufficient quantities of fresh specimens since the animals do not travel well over longer distances. However, if they are placed in filtered sea water immediately after capture, they recover relatively quickly.[1] p. 6

---

[1] I treated some of the specimens myself, and the remaining specimens were kindly supplied in the requested fixatives by the Heligoland Biological Institute. Live studies were conducted during my stay at the Norderney Research Station and particularly at the Max Planck Institute for Marine Biology in Wilhelmshaven. I am particularly indebted to Dr. A. BUCKMANN for allowing me to undertake my studies at his institute and for giving me ready access to its facilities. I am also grateful to Prof. Dr. H. BAUER for his many helpful suggestions.

Live studies were conducted in filtered sea water. Unstained houses and their structure were studied without difficulties in glycerin which is well-suited for these purposes because its refractive index differs considerably from that of the house substance. The details of a house thus treated can be observed very clearly.

Sections and prepared epithelia to be used for study purposes were always fixed in BOUIN's fluid as modified by ALLEN since this fixative produced the best results. In some cases, alcohol/glacial acetic acid in a ratio of 5:1 and Formol were used as fixatives.

The Feulgen reaction was used in most cases for staining the nuclei.

Toluidine blue in a 0.5% aqueous solution was used as stain for nucleoli. The nucleoli were examined without difficulty in the phase contrast microscope after applying the Feulgen reaction and subsequent staining with methyl green.

The HOTCHKISS reaction for polysaccharides[2] was sucessfully used in microchemical tests to determine the house substance. The secretion and the house substance very first hydrolyzed briefly with hydrochloric acid and then stained with toluidine blue according to the methods developed by LISON (1936) and HEMPELMANN (1940).

---

[2]This reaction is described in the footnote on p. 37 (source text).

III. Description of the Material

The specimens used in the present study were all collected in the mud-flats of the Deutsche Bucht.

Although the characters erected for Oikopleura dioica FOL in the descriptions of FOL (1872), LOHMANN (1933, 1896) and BÜCKMANN (1923, 1930, 1945) apply in general also to the species I have studied, there are some considerable differences as far as the structure of the intestinal tract and the characteristic number of the giant cells in FOL's oikoplasts are concerned. Since the description given by the above-mentioned authors and the results of the present investigations contradict each other in these two points, I am presenting below a summarizing overview of the morphology of my specimens of Oikopleura dioica FOL.

Body and tail of this appendicularian are two distinct parts. The tail is inserted between anus and gonads on the ventral side of the body. The anterior part of the body is covered by the oikoplastic epithelium (see Fig. 14), a thick, simple glandular epithelium which is easily distinguishable from the likewise simple covering epithelium on the posterior part of the body and the tail. The glandular epithelium secretes the cuticular house which encloses the entire animal (Fig. 4). p. 7

The mouth is located at the anterior end of the body and leads to the wide, thin-walled pharynx. The endostyle, a glandular groove (Figs. 5 and 6) is situated orally at the lower edge of the pharynx. Behind the endostyle and to the left and right of

the pharynx are the gill slits through which the water which has entered through the mouth is again discharged to the exterior. The anterior part of the pharynx carries two ciliated bands which extend along the side walls. These bands unite and form the medioventral ciliated band which continues rearwards into the oesophagus. The pharynx itself also merges posteriorly into the oesophagus and the latter opens on the left side into the left stomach lobe. The left stomach lobe is connected on its right side to the right stomach lobe from which arises the forward directed intestine. The intestine merges into the rectum which opens through the anus directly to the exterior.

All organs are located in the primary body cavity which is filled with a gelatinous mass. The gelatinous mass contains channels through which the blood passes in rhythmic pulses. The rhythmic pulsation of the blood is produced by the action of the wall of the pericardial sac which is located beneath the stomach. Connective-tissue cells, blood vessels and blood cells are lacking. A coelom is absent as well.

The gonads lie in the posterior part of the body cavity. While all other appendicularians are hermaphroditic-protandrous, Oikopleura dioica FOL has so far been the only species which is unisexual (LOHMANN 1933). An oviduct is always absent. The vas deferens appears only when the animals have almost reached sexual maturity.

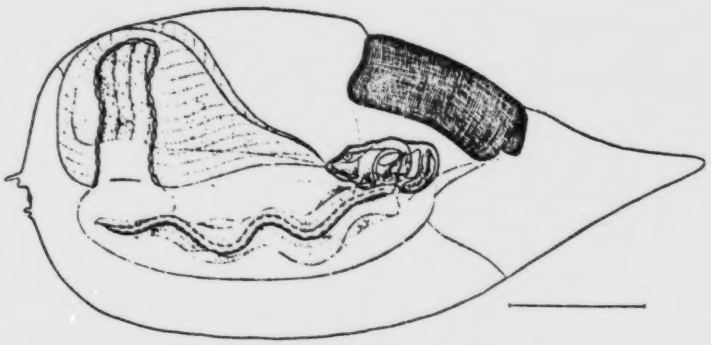

Fig. 4. Oikopleura dioica FOL within its house. Semi-schematic illustration drawn partly from life combined with shed houses. Scale 1000 µ.

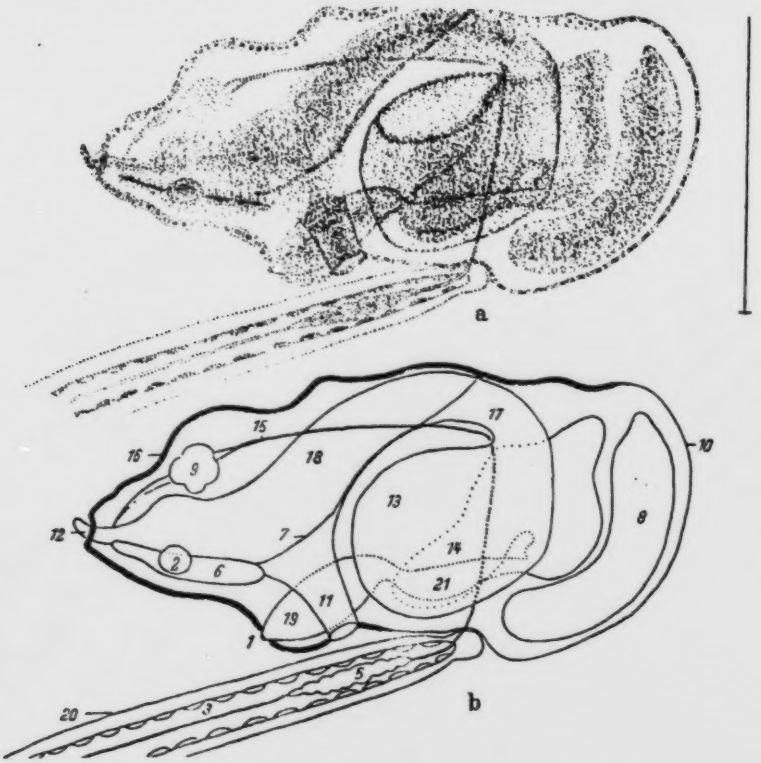

Fig. 5a and b. Oikopleura dioica FOL.

a - Body with tail base viewed from the left. Drawn from life. The transition from the oikoplastic epithelium of the anterior part of the body to the flat covering epithelium can be seen in the optical section.

b - Explanation. 1 - anus; 2 - oral gland; 3 - chorda; 4 - chorda cells; 5 - caudal ganglion; 6 - endostyle; 7 - ciliated band; 8 - gonads; 9 - cerebral ganglion; 10 - flat covering epithelium; 11 - gill slit; 12 - mouth; 13 - left stomach lobe; 14 - right stomach lobe; 15 - nerve cord; 16 - oikoplastic epithelium; 17 - oesophagus; 18 - pharynx; 19 - rectum; 20 - tail; 21 - intestine.

Scale 400 µ.

The nervous system is composed of a dorsal nerve cord and a cerebral ganglion. To the left of the ganglion lies the statocyst, and to the right the ciliated pit. The nerve cord has several thinner branches and extends to the anterior part of the body partly over, and partly lateral of, the pharynx. It turns downwards at right angles between the left and the right stomach lobe and from there extends the entire length of the tail. There the nerve cord bears a caudal ganglion and contains many ganglion cells. p. 8

The tail, which functions as a rudder, is compressed laterally. The dorsal side of the tail (viewed in relation to the position of the nerve cord) is turned to the left at right angles relative to the body. The chorda filled with the gelatinous mass extends ventrally from the nerve cord. The caudal integument is composed of flat epithelial cells. An elongate muscle band consisting of ten muscle cells one behind the other is located on either side of the tail beneath the integument. The dorsal and ventral margin of the tail is composed only of the flat epithelium of the integument.

An oral gland opening to the exterior is located on either side of the endostyle. "Exuvial bodies" are found in the house rudiment and the tail bears the two subchordal cells beneath the chorda. p. 9

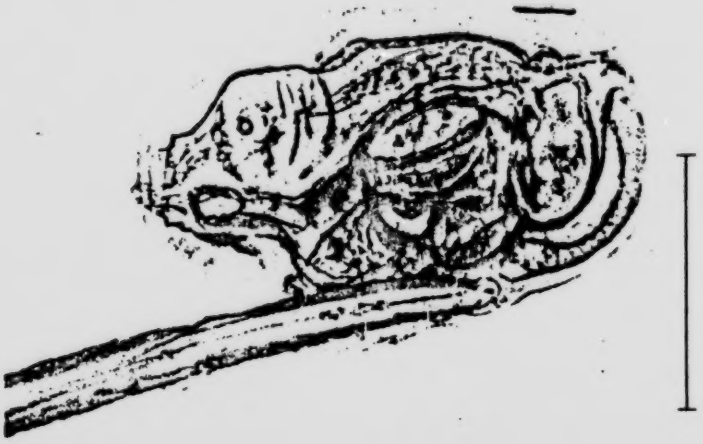

Fig. 6. Oikopleura dioica FOL. Immature specimen. Body with part of the tail. A house rudiment which has not yet expanded lies on the anterior part of the body. Micrograph of a live specimen. Scale 300 µ.

These last-mentioned characters are common to Oikopleura dioica FOL and several other species of the genus Oikopleura, which, according to LOHMANN (1933), constitute the labradoriensis group.

Oikopleura dioica FOL is completely identical with my material in all these points. Differences arise, however, in the outline of the intestinal tract and in the oikoplastic epithelium.

The mosaic-like structure composed of polygonal cells is a prominent feature of the glandular epithelium (Fig. 2), in which each cell has its own position and function. As previously mentioned, the most conspicuous of these cells are those groups of cells which are

responsible for the formation of special organs, such as the feeding apparatus and the incurrent grids, i.e. FOL's and EISEN's oikoplasts. They are composed of several large cells, the so-called "giant cells", which are surrounded by a certain number of smaller adjoining cells. FOL's oikoplasts in Oikopleura dioica FOL have been reported to have always nine giant cells (FOL 1872, LOHMANN 1933, BÜCKMANN 1923, 1930, 1945). By contrast, all the specimens from the mud-flats of the Deutsche Bucht possess only seven of the characteristic giant cells (Fig. 7). The number of filter-producing cells was always the same (namely 27), as was the number of giant cells, although earlier authors do not provide any precise details on this. It is assumed that the above-mentioned authors have based their investigations mainly on specimens from the Mediterranean, although BÜCKMANN's (1926) description of Oikopleura dioica FOL from the North Sea and the Baltic always refers to more than seven giant cells in FOL's oikoplasts. FOL (1872) and LOHMANN (1896, 1933) report the same number of cells for specimens from the Mediterranean. p. 10

The number and position of EISEN's oikoplasts in the specimens examined agree completely with the earlier description of Oikopleura dioica FOL.

LOHMANN (1933) and BÜCKMANN (1926) use only the left stomach lobe for identification and differentiation. My findings differ from those of these two authors who show the left stomach lobe to be rectangular, resting vertically on its narrow side. All of my live studies indicate that the pharynx is not appressed against the left stomach lobe which is only approximately square in outline (Fig. 8). THOMPSON's (1948) findings agree in this point with my conclusions.

However, it may well be that the differences in the shape of the left stomach lobe are due to different physiological conditions since particularly the intestinal tract is prone to change its shape. But this raises the question whether a feature as variable as the shape of the left stomach lobe should be used at all as a taxonomic character. For the time being, this problem remains unsolved.

The reason why I have stressed the above differences is to give a clear characterization of the material I have used in the present study. It will be up to a taxonomist to determine whether or not my specimens represent a geographic variant, for example, a subspecies. In any event, we can assume that Oikopleura dioica FOL has been accurately described by the earlier authors and that at least the number and location of the giant cells in FOL's oikoplasts are a characteristic feature. It should be mentioned, however, that in one instance BÜCKMANN (1923, 1945) has illustrated nine giant cells, in another instance (1926) ten, while LOHMANN (1896) shows eight and eleven giant cells, respectively. SALENSKY (1904) too, has found only seven giant cells in FOL's oikoplasts in Oikopleura rufescens FOL and in Stegosoma magnum LANGERHANS while LOHMANN (1896) and IHLE (1906, 1908) indicate eight giant cells in specimens of different origins. p. 11

In the following, I will use the designation Oikopleura dioica FOL only when referring to the specimens from the German Bight which I used in my studies. When referring to the species in FOL's original description (1872), I will identify this by adding the notation "nominate form".

Fig. 7. Oikopleura dioica FOL. FOL's oikoplasts with seven giant cells and three rows of 27 filter-producing cells each. Bouin-Allen, Feulgen reaction, methyl green. Micrograph after whole preparation. Scale 50 µ.

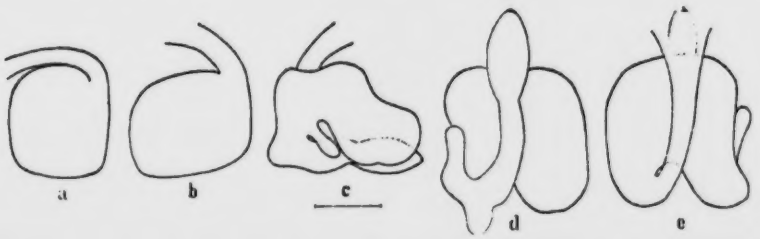

Fig. 8a-c. Outlines of intestinal tract in Oikopleura dioica FOL.
a - Left stomach lobe after BUCKMANN (1926).
b - Left stomach lobe of the form studied.
c - Right stomach lobe with blind sac, midgut and rectum.
d - Intestinal tract viewed ventrally.
e - Intestinal tract viewed dorsally.
Scale for b - e: 150 µ.

IV. Structure and Function of the Feeding Apparatus

The house of mature specimens of Oikopleura dioica FOL has a length of between 3500 and 4000 µ. About one-third of the inflated house is taken up by a complex filter system, the feeding apparatus, which is suspended in the anterior end of the house (see Fig. 4). This organ is used only for food collection and has no other function.

The feeding apparatus in Oikopleura dioica FOL differs only in its size from that of Oikopleura albicans LEUCKART. Although LOHMANN [1899 (1), 1899 (2), 1909] has clearly recognized its purpose and function, he has not adequately described its structure. The ratio between the size of the feeding apparatus and the size of the body in Oikopleura dioica FOL is the same as in Oikopleura albicans LEUCKART.

LOHMANN [1899 (1), 1899 (2)] has reported in detail on the mechanism involved in inflating the feeding apparatus. But here as well, a revision using modern techniques would be very useful. I was unable to p. 12
study the entire process of inflation of the house in Oikopleura dioica FOL and can therefore not comment on this process in detail. On the other hand, I was able to study inflated houses with their feeding apparatuses after they had been shed and I consider it necessary to describe here first the morphology of the feeding apparatus and its function.

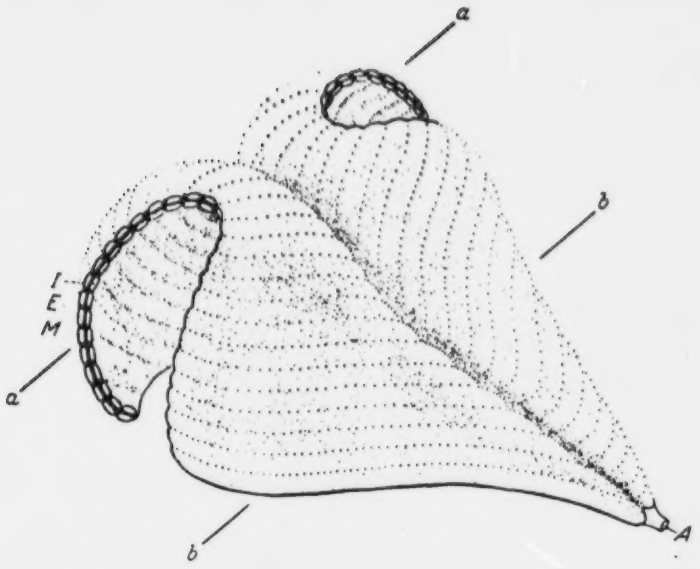

Fig. 9. Oikopleura dioica FOL. The feeding apparatus is a paired structure composed of two bilaterally symmetrical wings. The membrane covering the recess has been omitted from the drawing. - View of one side of the free margin with the incurrent orifices (I) and the excurrent orifices (E). The dotted lines indicate the filter tubes. M - median membrane; A - base of the mouth. Sectional planes a and b shown in the drawing (see Fig. 11).

A. Morphology of the Feeding Apparatus

This symmetrical structure composed of two wings can best be visualized as being formed by two layers which are joined along their lower edge over two-thirds of their length. The wings are arched outwards and are covered ventrally by a very thin membrane (Fig. 9). The cavity created in this manner is called the intermediate wing chamber.* An aperture for incurrent water is provided ventrally in the membrane of each wing.

*Translator's note: Literal translation.

The anterior third of each wing carries on the outwardly arched portion a recess which is also covered by a thin membrane (not illustrated in Fig. 9). Each of these two wings in turn is composed of three individual membranes, which form three superimposed membrane layers. The individual layers each enclose a space and are supported relative to one another by a system of supporting fibrils which, at the same time, increase the stability of the feeding apparatus. The supporting fibrils form 26 parallel tubes (Fig. 9) which correspond in number to a row of filter producing cells of FOL's oikoplasts. The tubes extend to the median line of the feeding apparatus where they open into a common longitudinal channel. The beginning of the tubes is shown by the tube openings which lie along the recess (see Fig. 9). The median membrane of the three-layer structure at the recess forms the above-mentioned thin cover membrane, while the membranes lying above and below this cover membrane extend only as far as the recess (Fig. 12). The view of Fig. 9 is obtained when we remove, from the recess, the median membrane which functions as a covering membrane at that point, thereby revealing the free margin of the three-layer wall which is supported by fibrils.

p. 13

p. 14

The supporting fibrils separate the individual channels. Such a system of channels extends <u>beneath</u> the median membrane to the median line where the two wings are interconnected and forms here a common channel which extends to the posterior end of the tapering wings. The second channel system runs <u>above</u> the median membrane parallel to the first system and contains a filter system in each of its channels. These channels, the so-called filter tubes, also open into the common main channel (Fig. 11a and b). The <u>ingestion orifices</u> lie within the so-called cover membranes

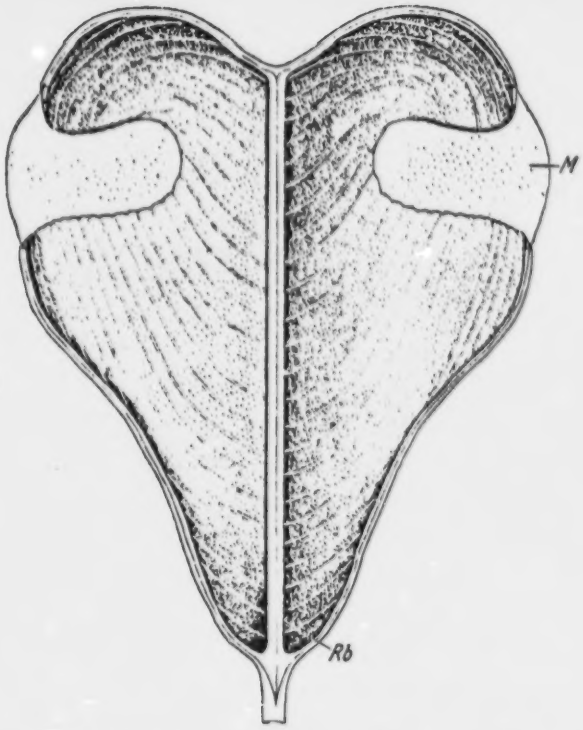

Fig. 10. Oikopleura dioica FOL. Ventral view of the feeding apparatus. The margins of the recess are covered by the median membrane (M). The raised filter floor (Rb) extends along the median line. The tubes indicate the course of the incurrent channels. Omitted from the drawing is the cover membrane which does not completely close the feeding apparatus ventrally. Semi-schematic representation.

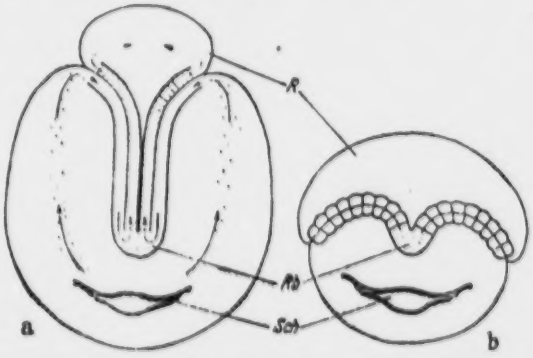

Fig. 11a and b. Oikopleura dioica FOL. Schematic frontal sections of the feeding apparatus. See Fig. 9 for location of sectional planes.

a - Ideal section of the intermediate wing chambers. The arrows indicate the direction of the water current containing the plankton. The excurrent water then enters the dorsal chamber R.
S - caudal chamber
Sch - tail
Rb - filter floor.

b - Ideal section of the posterior third of the feeding apparatus where several oblique tubes are sectioned. The incurrent channels are situated ventrally, while the excurrent channels are superimposed on them.

at the point where the wing recess starts, while the egestion orifices (Fig. 12) are superimposed on them, but lie outside of the membrane. The cover membrane closes the intermediate wing chamber ventrally and separates it from the caudal chamber of the house. Fig. 4 does not show all of the chambers since the delicate nature of their membranes made it impossible to determine their precise outline. p. 15

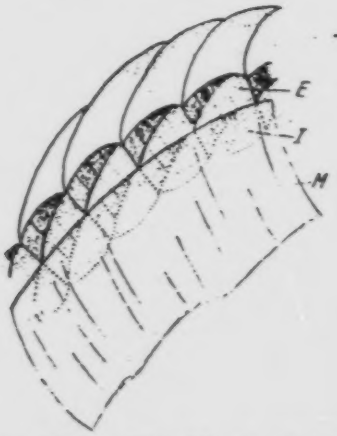

Fig. 12. Oikopleura dioica FOL. Detail of the free margin of the feeding apparatus with incurrent and excurrent orifices. The two orifices are separated by the median membrane which prevents a mixing of filtered and unfiltered water.
I - incurrent orifice
E - excurrent orifice
M - median membrane.

B. Function of the Filter System

Movement of the tail in Oikopleura causes water together with nannoplankton to enter through the two orifices left open in the cover membrane and to pass into the intermediate wing chambers. The continuously generated pressure forces the water through the numerous ingestion orifices from where it passes through the tubes of the channel system and reaches the common main channel. Here the suspended

particles are strained from the water. Since the animal opens its lip only occasionally, which opens the orifice to the main channel connected to the mouth, the inflowing water in the main channel comes almost to a standstill and, during this short time, much of the nannoplankton settles to the bottom of the main channel, i.e. on the so-called "filter floor" (see Fig. 11a and b). The water itself flows through the outer channel system, the so-called filter tubes, where the remainder of the plankton and of the suspended particles are strained by the transverse fibrils, and then flows parallel to the inflow direction back to the exterior. The water enters the dorsal chamber of the house through the egestion orifices of the wings and passes from there through the excurrent orifice of the house, together with the gill water, to the exterior. From time to time, the animal uses suction to ingest the plankton material strained from the water and collected on the filter floor.

After food has been collected in this manner for a certain period of time, the filter apparatus ceases to function because the filter tubes become clogged. As a result, the animal sheds its old house and develops a new one. The previously held view, however, (LOHMANN and BÜCKMANN 1926, LOHMANN 1933) that the animal cannot collect food when it has shed its house is not totally correct. I frequently observed animals ingesting food directly through the mouth even when they had no feeding apparatus and I also noted a considerable amount of plankton on the endostyle. Moreover, the stomachs of those animals which had not developed a new house were always full when they were kept in small glass vessels over relatively long periods of time. Tests using ink particles proved that food was ingested directly through the mouth. The

p. 16

food particles ingested directly through the mouth were much larger than the particles strained in the feeding apparatus because the mouth of the animal is relatively large. When living in their natural environment, the animals also ingest food without their feeding apparatus and house because the stomach contents very frequently contain diatom shells, etc., which are too large to even pass through the grids of the incurrent orifices (Fig. 13).

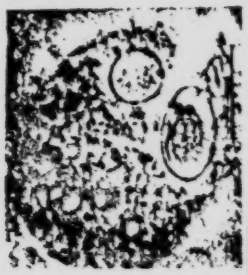

Fig. 13. Oikopleura dioica FOL. Large diatom shells and other foreign particles in the intestinal tract. The shells are too large to pass through the grids of the incurrent orifices. Their presence can only be attributed to direct ingestion through the mouth. Micrograph, scale 10 μ. Compare the mesh size of the incurrent grids.

It is obvious that the food entrapped in the filter apparatus is collected much faster and more conveniently than by direct ingestion through the mouth. A more important aspect is the relation between the size of the food particles and the rate of digestion. As is well known, smaller food particles are more readily digested by the digestive enzymes than correspondingly larger particles. Accordingly, digestion and resorption involve a certain time factor. A considerable amount of

nutrient substance must be ingested per unit time to enable the animal to produce the building material for such a large number of houses, and the smallest planktonic organisms are preferred as they are more rapidly digested. In addition, only part of the ingested food is converted into building material for the house since some of the food is required to supply the necessary energy for locomotion. Although the animal is capable of ingesting food even without the feeding apparatus, and considerable amounts of food at that, as we have seen, it is no doubt the presence of the feeding apparatus which ensures the life of the animal over a relatively long period of time.

In summary, therefore, it may be said that the complex, yet convenient ingestion of large amounts of minute plankton within a very short time facilitates digestion and resorption considerably. At the same time, sufficient amounts of nutrient substances are obtained to produce the large amounts of secretions which are continuously required for the formation of new houses. Food ingestion and house formation are interdependent and constitute a closed physiological system.

V. The Oikoplastic Epithelium

p. 17

A glandular pavement epithelium extends dorsally over the entire anterior part of the body all the way to the gonads and ventrally to the tail base, except for a wedge-shaped portion behind the anus (Fig. 14). The thickness of the polygonal cells in the epithelium varies in the individual regions. Whereas the cell layers are very thick in the

central region of the body, on the dorsum and in the mouth region, the ventrum and the stomach region have only a thin layer of very flat cells. Mouth, gill slits and anus interrupt this mosaic-like arrangement of the cells.

Number and arrangement of these glandular cells are always the same. Several precise counts produced 2,290 ± 30 oikoplasts for Oikopleura dioica FOL. They are rich in plasma and have a round, oval or elongate, compact nucleus, depending on the shape of the cell in which it is located. While other species very often have branched or lobed nuclei (Fig. 15) (LOHMANN 1933, KLAATSCH 1895), this is not the case in Oikopleura dioica FOL. The nuclei are not branched either in the live specimen or after treatment with Bouin-Allen fixative. On the other hand, I found branched nuclei even in the present species when fixatives were used which contained mercuric chloride or osmic acid. I am unable to ascertain whether the branched nuclei found by earlier investigators are artifacts as well. p. 18

The nuclei of the oikoplasts are very rich in chromatin (Fig. 16) and much larger than the cell nuclei of the other somatic tissues (Fig. 17). It may lead us to assume that the oikoplasts are polyploid. Since, on the one hand, the differences in size of the oikoplasts are extreme, and on the other there are transitions from the largest to the smallest cells, it may well be assumed that the cells in the oikoplastic epithelium belong to different levels of polyploidy. However, the studies have shown that the same amount of chromatin is present in all cells.

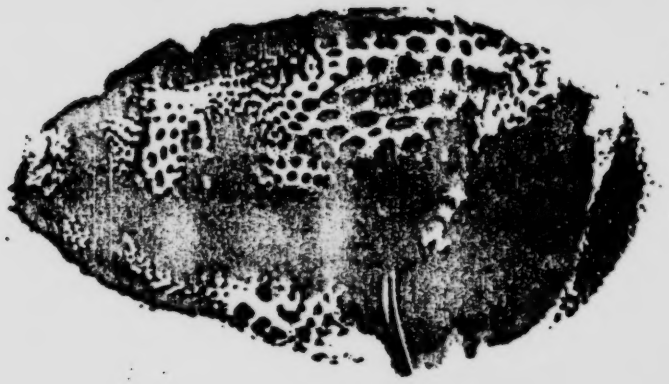

Fig. 14. Oikopleura dioica FOL. Left side of the body with oikoplastic epithelium. Bouin-Allen. Feulgen, methyl green. Micrograph of a whole preparation. Scale 300 µ. Compare the minute cell nuclei of the pharynx with those of the oikoplastic epithelium.

Abb. 15.

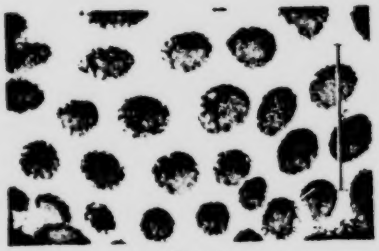

Abb. 16.

Fig. 15. Oikopleura albicans LEUCKART. Branched nuclei from the dorsal region of the oikoplastic epithelium. Specimen treated with carminic acid and counterstained with iron hematoxylin. Scale 150 µ.

Fig. 16. Oikopleura dioica FOL. Nuclei from the oikoplastic epithelium; nuclei rich in chromatin. Bouin-Allen, Feulgen. Micrograph. Scale 90 µ.

The only difference is that chromatin is dispersed in the larger nuclei, while it is crowded into a very small space in the smaller nuclei. The presence of heterochromatin gave an indication of the level of polyploidy.

> We examined the diploid genome of normal somatic cells in order to establish the level of polyploidy. Fig. 18 shows a normal cell division in the ectoderm of the larva of Oikopleura dioica FOL. This type of cell was used because cell divisions are relatively rare in the adult animal and, in addition, the nuclei in the cells of the intestinal tract are very small. Four smaller and two somewhat larger chromosomes (see scale added in Fig. 18) lie near the poles of the cell. Three haploid chromosome tetrads are present during oogenetic and spermatogenetic meiosis (Fig. 19).

Cell division did not occur in the oikoplastic epithelium, and the level of polyploidy can therefore only be conjectured by determining the amount of heterochromatin present. The diploid cell nuclei of the intestinal tract have always one heterochromatic constituent. By contrast, the nuclei of the oikoplastic epithelium have always two heterochromatic constituents, although they cannot always be identified (glandular function of the cell!). It follows that the cells of the oikoplastic epithelium are probably at least tetraploid, independent of their size. p. 19

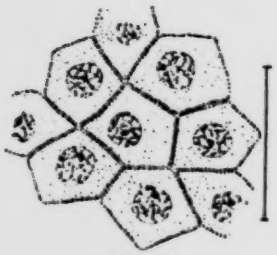

Abb. 17.

Abb. 18.

Abb. 19a. u. b.

Fig. 17. Oikopleura dioica FOL. Group of cells from the pharynx. Compare the size of the nuclei of these intestinal cells with that of the oikoplasts. Scale 20 µ.

Fig. 18. Oikopleura dioica FOL. Anaphase in the ectoderm of the larva. Scale 10 µ.

Fig. 19a and b. Oikopleura dioica FOL.

a. Equatorial plate from a first-order spermatocyte. Carminic acid preparation.
b. Oogenetic meiosis with three chromosome tetrads. Scale 10 µ.

Abb. 20.

Abb. 21.

Fig. 20. Oikopleura dioica FOL. Isolated large oikoplasts from the dorsal region (Fig. 3; i). Many nucleoli are visible in the nuclei. Bouin-Allen. Toluidine blue; counterstained with iron hematoxylin. Micrograph. Scale 20 µ. Compare with Fig. 21.

Fig. 21. Isolated small oikoplasts from the pharynx region (Fig. 3; b) with less nucleolar substance corresponding to the size of the nucleus. The nucleoli are agglomerated in some of the cells. Bouin-Allen. Toluidine blue; counterstained with iron hematoxylin. Micrograph. Scale 20 µ. Compare with Fig. 20!.

However, it may well be that several heterochromatin elements are agglomerated in the oikoplast nuclei and that the level of polyploidy is, therefore, even higher. p. 20

The number of nucleoli in the nuclei varies considerably. It can be assumed that their number is indicative of the different functions of the various cells. It is also noted that the number of nucleoli depends on the size of the cell and of the nucleus. It increases with increasing cell size (Figs. 20 and 21). The nucleoli change during the secretion process and assume very peculiar shapes.

VI. The Intracellular Secretion Processes

A. General Secretion Process

In general terms, secretions are chain reactions. The secretion process of any organ consists of a closed sequence of individual reactions which merge continuously with one another. It is for purely practical purposes that, in the following, individual phases will be described separately and apparently independently in order to focus on individual secretion products from the overall process and to give a clear picture of the individual stages. Secretion is never the work of an individual cell constituent but always the result of harmonious secretory activity of many structures and substances in a cell.

The amount of secretion which an individual oikoplast in Oikopleura dioica FOL must produce in the course of its life span is enormous, and the oikoplast and its nucleus as the centre which controls the secretion

process must, therefore, be capable of meeting that challenge. The amount of secretion elaborated by the oikoplasts is particularly impressive when we realize that the animal produces 5-6 houses in a 24 hour period and has a life span of 1/2 year [according to SEELIGER 1893 (2)].

According to CASPERSSON (1936), the nucleic acid component changes considerably during the various functional phases of a glandular cell. Nucleic acid is stored in relatively large amounts during the resting stage and disappears after the secretion has been elaborated. This indicates a direct involvement of the nucleus in the chemical processes within the cell, and it seems that this applies to some extent also to the oikoplasts in Oikopleura dioica FOL. I was unable to discern an actual resting stage in the secretory oikoplasts since the individual secretion phases are not only continuous, but, in many instances, occur parallel to one another. Nevertheless, I did note that with the secretion of the house the heterochromatin disappeared.

B. The Individual Phases

p. 21

G. CHR. HIRSCH (1938) generally distinguishes four phases during secretion. On the basis of my own observations in Oikopleura dioica FOL, I decided to divide the secretion process into only three phases.

The production of prosecretions, the formation of the final secretion, and its discharge occur side by side. This means that the individual

secretion phases merge with one another and that formation and discharge occur parallel to one another. This peculiarity makes it considerably more difficult to study a particular phase than is the case in a normal secretion process.

a) Formation of Prosecretions.

The nutrients are certainly supplied in dissolved form since the nutrient substances become visible only when they reach the cell boundary facing the interior of the body. They deposit at the cell boundary as solids and form a kind of precursor of the subsequent secretion. They are present in the form of small granules, sometimes as small agglomerations, sometimes as larger clusters (Fig. 23a). These substances stained only with the Hotchkiss reaction and are therefore polysaccharides (Fig. 22).

Proteins and fats were not identified. Protein substances, which are required for the synthesis of the final secretion, are not formed until later in the process. Fats were not identified at any stage during the secretion of the house. It should be mentioned briefly at this point that the nucleoli are apparently responsible for the formation of the protein substances and that the nucleoli change their shape at that stage. p. 22

The small polysaccharide granules, which have been deposited on the inner cell wall, increase in size during the secretion process and start to migrate gradually through the cell. The increase in size is probably due to the deposition of additionally supplied nutrients while the small granules act, as it were, as condensation nuclei (Fig. 23b).

Whether the nutrients deposit in shell-like layers is difficult to determine because these granules are very small.

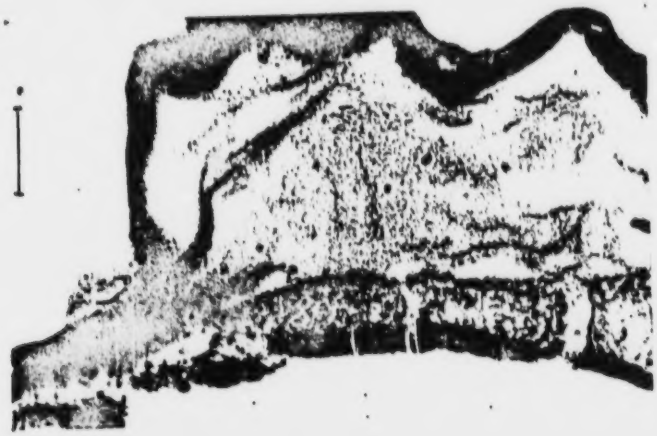

Fig. 22. Oikopleura dioica FOL. Cross section of the oikoplastic epithelium with house rudiment near the dorsal cells. The cells contain many small polysaccharide granules along the inner cell boundary. Bouin-Allen, Hotchkiss reaction. Micrograph. Scale 10 μ.

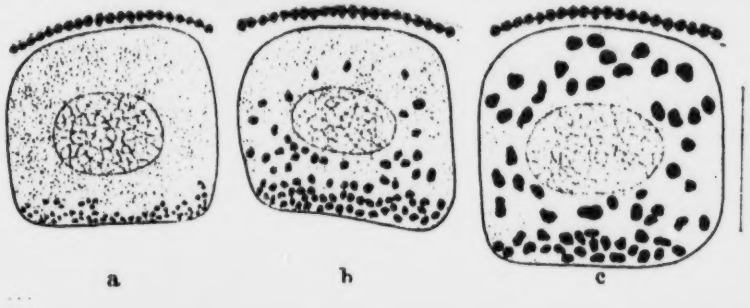

Fig. 23a-c. Oikopleura dioica FOL.

a. Cell from the oikoplastic epithelium, with prosecretion. Numerous small polysaccharide granules are present on the inside of the cell boundary facing the interior of the body. The wall of the house rudiment is shown above the cell.

b. Oikoplast during elaboration of secretion. The polysaccharide granules have increased in size and have started to migrate through the cell. The wall of the old house rudiment is shown above the cell.

c. Cell from the oikoplastic epithelium. The polysaccharide granules have reached their full size and are distributed throughout the cell.

Scale 10 μ.

The polysaccharide granules continue to increase in size as the process continues and spread throughout the cell (Fig. 23c). At the same time, the nucleoli in the nucleus have changed their shape. While they were small and spherical at the beginning of the secretion process, they have meanwhile increased in size and are gradually becoming ribbon-shaped. Their change in shape becomes even more pronounced in the second phase (Fig. 24a and b). If we assume that the nucleoli are responsible for the formation of protein substances, it is logical to conclude that the larger cells (giant cells, dorsal cells) which elaborate more secretion also contain more nucleolar substance. Each individual cell contains, therefore, the necessary number of nucleoli depending on the amount of secretion it produces. The number of nucleoli ranges from 3 to 50.

The nuclei in mature animals did not change noticeably in size during the secretion process. It appears that uptake and discharge of substance by the nucleus occur continuously, i.e. not only while secretion is discharged but also during the refractory phases. Uptake and discharge balance each other. We were unable to determine p. 23 morphologically a phase of increased nuclear activity. Heterochromatin can be identified (more or less) in all intracellular phases of secretion and disappears only when the secretion is discharged to the exterior to form the wall of the house. Presumably, only the thymonucleic acid component changes at that time.

Fig. 24a and b. Oikopleura dioica FOL.
a - Cell from the oikoplastic epithelium with ribbon-shaped nucleolus at the time the final secretion is formed. Bouin-Allen, toluidine blue; counterstained with iron hematoxylin. Micrograph of a teased-out preparation.
b - Drawing from Fig. 24a.
Scale for a and b: 10 µ.

---

b) Formation of the Final Secretion

The last phase involves the elaboration of the secretion with its new, specific properties. The secretion is a more or less homogeneous substance composed of polysaccharide granules and the protein substances formed by the nucleoli (Figs. 25 and 26).

The final secretion becomes visible when the specimen is first subjected for a short period to hydrolysis in 1 N hydrochloric acid and subsequently stained with a solution of toluidine blue α according to LISON.

A much greater contrast was obtained when the same toluidine blue solution became inadvertently infected with a mould for a relatively long time (about four weeks). Presumably, this caused the solution to mature.

The micrograph obtained after the toluidine-blue treatment showed the following: The final secretion surrounds the nucleus in the cell as moniliform structures, with the granules arranged in strand-like fashion. The ribbon-shaped secretory strands lie a certain distance away from the nucleus, as do the polysaccharide granules. The amount of secretion is always proportional to the cell volume.

The microchemical analysis showed that the final secretion consists of two secretion components. Polysaccharide was again identified by means of the Hotchkiss reaction, while a small amount of protein was determined with BERG's Ninhydrin reaction.

The moniliform secretory strands are deposited from the inside along the outer cell boundary as the intracellular processes of secretion continue (Fig. 27b). The outer boundary layer was formed as a distinct membrane when the secretion process of the previous house rudiment was completed and this "cell boundary" is now being incorporated into the new house rudiment. p. 24

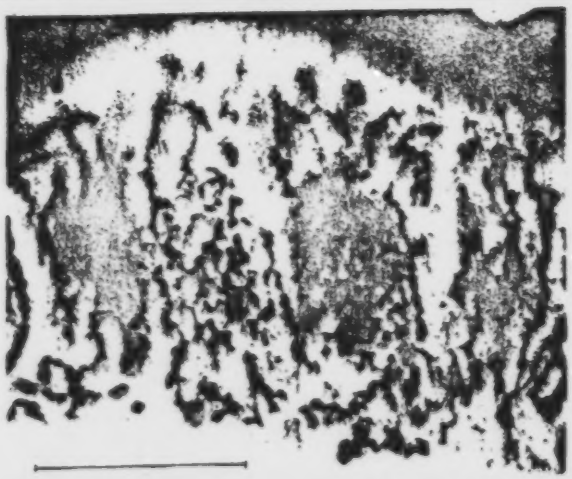

Fig. 25. Oikopleura dioica FOL. Sagittal section of the oikoplastic epithelium. The secretion lies in the cells as moniliform strands. The individual strands of secretory granules are discernible. Bouin-Allen, toluidine blue. Micrograph. Scale 10 µ.

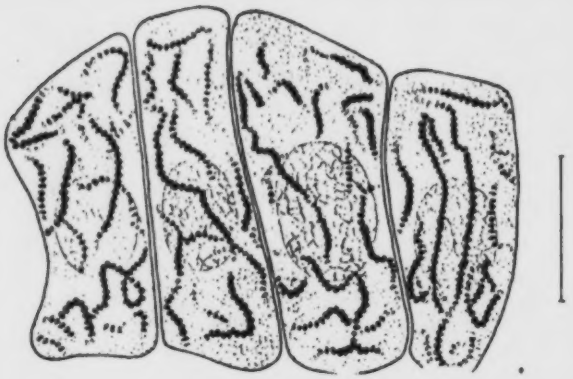

Fig. 26. Drawing from Fig. 25. Scale 10 µ.

---

c) Secretion of the Secretory Strands

The outer "cell boundaries" must be regarded as products of the previous secretion process. They are extremely delicate and thin membranes.

The secretory strands deposit along these cell boundaries from the inside and form a layer which is now covered on the inside by a delicate membrane similar to the cell boundary (Fig. 27c). A second similar membrane develops below this membrane and forms the new cell boundary (Fig. 27d). Since the formation of secretion is a continuous process, the cell is already depositing a new secretion layer below the new cell boundary so that a new house can immediately be secreted when required (Fig. 27d and e). The house rudiment can now separate from the epithelium; the oikoplasts appear protected towards the exterior by the thickened cell boundary (Fig. 28)[1]. p. 25

---

[1]Micrographs have not been included here nor in several other instances. They are, however, available at the Zoological Institute in Darmstadt.

The secreted house rudiment is bounded on the outside by the former cell boundary which has now become the "outer boundary membrane". The secretory strands lie underneath. Their string of secretory granules is clearly discernible and they are bounded on the inside by the "inner boundary membrane" (Fig. 29).

LOHMANN (1896) illustrates fibrils in the wall of the house (Fig. 30) which I was unable to detect. It is, however, very likely that the secretion processes in Oikopleura labradoriensis LOHMANN are essentially the same as in Oikopleura dioica FOL and that the final product is also the same.

The secretory strands do not decrease in number during and even after secretion of a house rudiment. This is so because all three phases occur simultaneously side by side and secretion is constantly being elaborated. p. 26

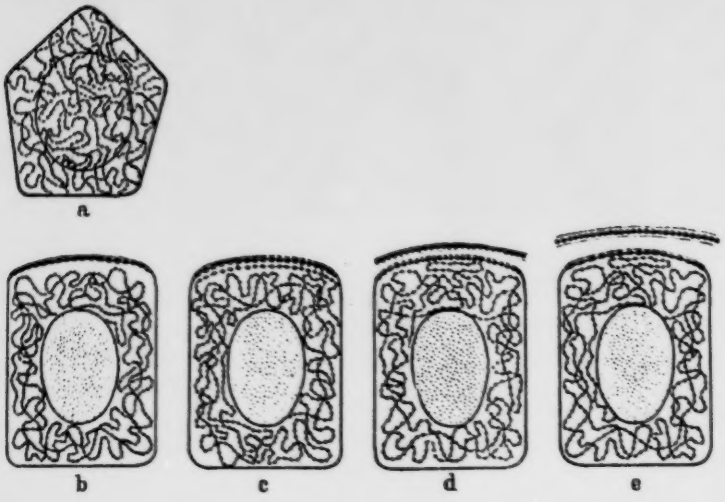

Fig. 27a-e. Schematic illustration showing the secretion process of the house.
a - Surface view of individual oikoplast.
b - Secretory strands are deposited along the outer cell boundary.
c - Formation of a delicate membrane beneath the secretion layer and formation of a new cell boundary.
d - Separation of the wall of the house and deposition of new secretory strands along the new cell boundary.
e - More advanced stage.
b - e cross-sections.

Fig. 28. Oikopleura dioica FOL. Sagittal section of three oikoplasts. Separation of the house rudiment. The secretory granules are discernible in the wall of the house. Secretory strands lie within the cell. Scale 10 µ.

Abb. 29.

Fig. 29. Oikopleura dioica FOL. Fine structure of the house wall shortly after secretion. The secretory strand is enclosed by the two boundary membranes. Cross section. Scale 10 µ.
a - Outer boundary membrane.
b - Inner boundary membrane.
c - Secretory strand.

Abb. 30.

Fig. 30. Oikopleura labradoriensis LOHMANN. Cross section of the house wall. Without scale (after LOHMANN 1896).

The extracellular processes occurring after the house rudiment has been secreted are all of a purely structural nature and will be described separately in one of the following chapters.

In summary, we can see that the oikoplasts of the secretory epithelium are polyphasally functioning cells which are integrated in the glandular processes in such a fashion that they are apparently capable of supplying secretion throughout the life of the animal without requiring replacement.

As previously mentioned, I have been unable to determine any change in shape of the nuclei in the oikoplastic epithelium. I therefore would like to discuss briefly KLAATSCH's (1895) statement that he noticed changes in the nucleus in the oikoplasts of Oikopleura cophocerca GEGENBAUR during the formation of the house. I also found nuclear structures similar to those illustrated in KLAATSCH in Oikopleura dioica FOL after poor fixation, while all nuclei in live specimens are spherical or oval. On the other hand, a few branched nuclei are always present in the oikoplastic epithelium of Oikopleura albicans LEUCKART. I would not associate this branching of the nuclei with the secretion process, particularly since KLAATSCH (1895) does not mention the secretion process at all. Rather it would seem that the nuclei in Oikopleura cophocera GEGENBAUR are normally lobed and branched. Unfortunately, other authors do not provide any details on this point. p. 27

VII. The Extracellular Processes during Formation of the House

It can be assumed that the secretion processes resulting in the formation of a house involve the action of enzymes, while the processes following the secretion of the house are believed to be purely physical in nature. Since the surface area of the completely inflated house is about 100 times the surface area of the secreted house rudiment, we can safely assume that the incorporation of water molecules in the discharged secretion produces a swelling action in certain directions. We can visualize that as a result, the house rudiment expands until it reaches the size of the fully inflated house with feeding apparatus. Since very little is known so far about the processes involved, I can only present my theory without being able to interpret the underlying mechanism.

A. Development of the Final Fine Structure of the House

The products of secretion from the individual cells fuse with each other and retain seams that can be detected by staining procedures (precipitation staining) until the time when the house rudiment separates from the epithelium and has not yet noticeably expanded. The varions parts of the house delimited in that way still show their origin from the cells from which they were elaborated (Fig. 31). At this point, the house is at a stage which resembles that of the chitinous cuticulae in arthropods. For example in the daphnids, the shield also shows a polygonal tesselation.

As the house expands, these mosaic-like structures are no longer congruent with the associated cell surfaces, yet maintain a similar configuration with them until the fusion seams have expanded to a point where they are no longer discernible.

I was able to examine the fine structure of the secretion before and after expansion of the house wall. Prior to expansion, the secretory granules are arranged in the house wall in mutual contact with one another and appear somewhat swollen compared with those inside the cell (Fig. 29). As expansion progresses, more or less uniform gaps develop between the secretory granules, which are now isolated from each other. No changes have been observed on the outer and inner boundary membranes. Similarly, the secretory granules as such do not undergo any further changes in shape and size.

Based on these findings, we can draw some conclusions about the expansion mechanism involved. Expansion is presumably caused by systematic integration of water molecules in the expandable boundary membranes. p. 28

B. The Formation of the Feeding Apparatus

The elaboration of the secretion precursors and of the final secretion is the same both in FOL's and EISEN's oikoplasts and in the other oikoplasts, but the secretion processes differ considerably. While the cells in the oikoplastic epithelium which are directly involved in the formation of the house function in a uniform pattern, three different types of cells in FOL's oikoplasts are responsible for secreting three different structures.

a) Secretion Mechanism of the Giant Cells

During the secretion process, the giant cells secrete a gelatinous cushion which serves as a stable support for the filter system, as was previously reported by LOHMANN [1899 (1) and 1933] (Fig. 32). The cushion itself disappears later when the feeding apparatus has been completely secreted. Only the boundary membranes of the cushion are left behind and form a wall which becomes appressed from the inside against the filter system, thereby increasing its stability. This finding is contrary to LOHMANN (1933) who assumed that the cushions disappear completely. The wall formed in this manner does not show any fibrils and presumably the gelatinous cushion is not secreted in the same rigid sequence as the house wall since it is completely structureless. The gelatinous cushion expands in proportion to the growth of the filter system, i.e. it increases its surface area to accommodate the filters until they reach their final size. p. 29

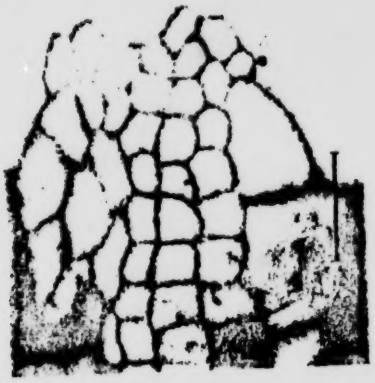

Fig. 31. Oikopleura dioica FOL. Part of a house rudiment removed from the oikoplastic epithelium. The house was still firmly attached to the epithelium and had not yet expanded. The polygonal tesselation of the cell boundaries has been made visible by precipitation staining. Bouin-Allen, iron hematoxylin. Micrograph. Scale 15 µ.

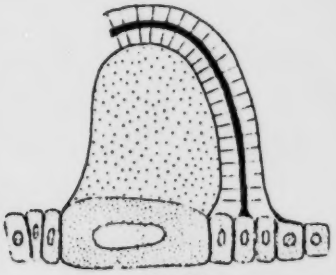

Abb. 32.

Fig. 32. Schematic representation of the secretion process taking place in FOL's giant cells. The structureless gelatinous mass above the giant cell disappears after the filter system has been secreted. Only its boundary membrane is left behind, forming a wall which becomes appressed from the inside against the filter system.

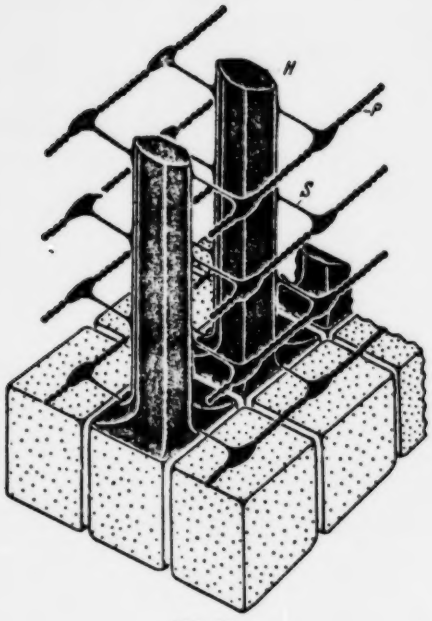

Abb. 33.

Fig. 33. Oikopleura dioica FOL. Schematic representation of the secretion mechanism of the filter producing cells. The main fibrils (H) emerge from the median row of filter producing cells, while primary and secondary fibrils (P and S) arise from the adjoining lateral rows. Note the moniliform structure of the primary fibrils.

---

b) Secretion Mechanism of the Filter Producing Cells.

FOL's oikoplasts are responsible as a whole for the formation of the feeding apparatus (Fig. 43a-c). The seven giant cells are followed posteriorly by three longitudinal rows of 27 small cubical cells each (Fig. 43a; f). These are the filter producing cells which secrete a complex system of fibrils. Their secretory activity is more varied and specialized than that of all other cells of the oikoplastic epithelium. All three longitudinal rows of cells are involved in the production of the filter system (Fig. 33) which requires an extremely coordinated interaction of these cells in order to produce a well-functioning structure.

LOHMANN (1933) assumed that the transverse and longitudinal fibrils were formed by the boundary surfaces between the median row of cells and the adjoining cells. I cannot confirm this assumption. According to LOHMANN, the fibrils would have to be secreted above the cell boundaries which are perpendicular to the body axis. Rather, it is the free cell surfaces on the outside of the body which secrete the filter elements (Fig. 34), as is the case with all other cells of the oikoplastic epithelium. p. 30

Following the pattern of the other oikoplastic cells, the filter producing cells in the median row secrete a dorsoventrally flattened main fibril on each cell surface (Figs. 33 and 35). A new cell boundary is formed only when the secretion of the fibril is completed. The secretion mechanism of the main fibril is as follows:

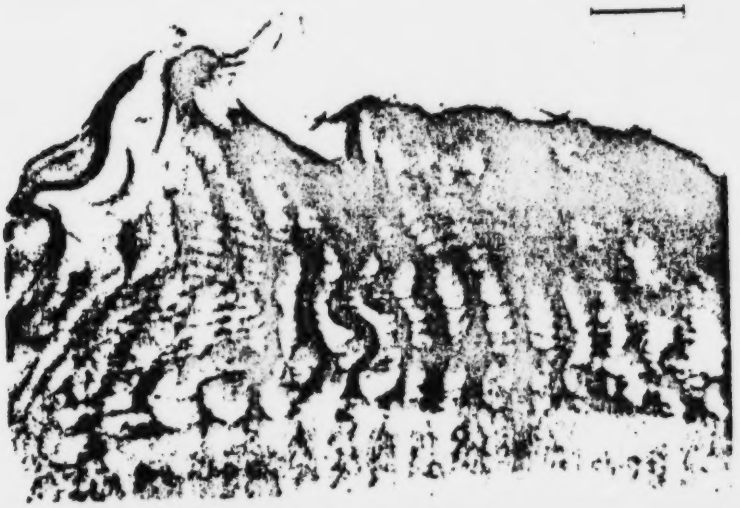

Fig. 34. Oikopleura dioica FOL. Emergence of the main fibrils from the cells of the median row of filter producing cells. The primary fibrils extend perpendicular to the main fibrils. The secondary fibrils are visible as dark dots in the top view. Bouin-Allen, toluidine blue, cross section. Micrograph. Scale 10 µ.

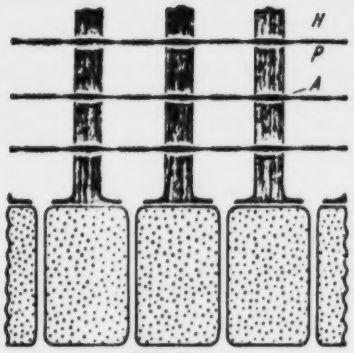

Abb. 35.

Abb. 36.

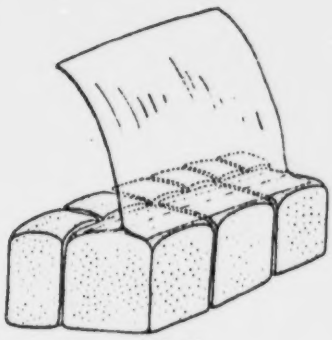

Abb. 37.

Fig. 35. Drawing to Fig. 34. Schematic representation.
H - Main fibrils.
P - Primary fibrils.
A - Attachment points of secondary fibrils.

Fig. 36. Oikopleura dioica FOL. Section of the secreted filtering fibrils perpendicular to the main fibrils. Semi-schematic representation. Lettering as in Fig. 33. Scale 10 µ.

Fig. 37. Oikopleura dioica FOL. Schematic representation of the secretion mechanism of the boundary cells. They secrete a cover membrane on their outer surface. This membrane is then deposited on the outside of the filter tubes.

When the secretions emerge from the cell body, they migrate from the peripheral regions of the cell to a central zone where they form a strong fibril. This main fibril is connected to the secretions of the anteriorly and posteriorly adjoining cells only by a delicate bridge which is presumably secreted from these adjoining cells (Fig. 36).

Each of the two outer rows of filter producing cells secretes longitudinally a primary fibril from the cell surfaces, whereupon the individual secretion products from these cells become fused. The moniliform structure of the secretion is still discernible in these fibrils. Very short secondary fibrils are secreted in the transverse direction and attach to the main fibrils, thereby forming the connecting

bridges. The amount secreted by each cell to produce a primary and a secondary fibril is so small that a large number of these fibrils can be formed successively at regular intervals. Up to 40 have been counted. Although secretion is similar to that of the oikoplasts which secrete the house, it is restricted to a small area on the cell surface (see Fig. 33) and occurs rhythmically at very short time intervals. p. 31

The secondary fibrils fuse with the main fibrils at regular intervals. This synchronous activity to the left and right shows the precision with which the individual cells interact during the secretion processes to produce exactly the right structure at the right time. These cellular activities occur at precisely the right time and the right place down to minute details, thus ensuring that the various "building blocks" are secreted in a coordinated fashion.

c) Secretion Mechanism of the Boundary Cells

The function of the boundary cells is to produce a cover membrane which is deposited on the outside of the filter tubes. The elongate, almost fusiform cells secrete a thin membrane perpendicular to their surface in the same manner as the main fibrils of the filter elements are secreted. This thin membrane grows at the same rate as the filter tubes and is eventually firmly bonded to the feeding apparatus (Fig. 37). p. 32

C. The Formation of the Incurrent Grids

Large oikoplasts surrounded by small cells are also situated on the lateral surfaces of the oikoplastic epithelium, very close to the

tail base. These are EISEN's oikoplasts, which are present on either side and are responsible for forming two incurrent grids interposed in the dorsal posterior half of the house (Fig. 43a–c). These oikoplasts are composed of seven giant cells along whose proximal margin extends a row of 17 very small cells which may be referred to as "microcells" when compared with the giant cells. p. 33

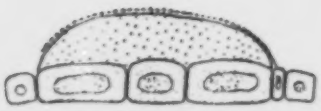

Fig. 38. Oikopleura dioica FOL. Schematic representation of the secretion process of EISEN's giant cells. The structureless gelatinous cushion covering the giant cells disappears later. The remaining boundary membrane then forms the covering membrane of the incurrent grids.

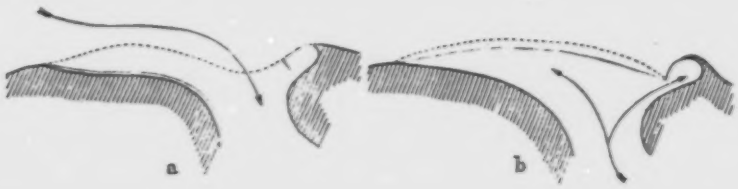

Fig. 39a and b. Oikopleura albicans LEUCKART.
a Ideal section of an incurrent grid with inflow of water.
b Ideal section of a grid with outflow of water. The function of the covering membrane is evident from the drawing (after LOHMANN 1899).

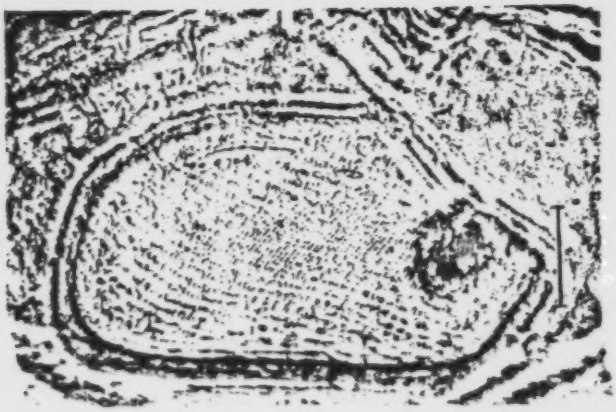

Fig. 40. Oikopleura dioica FOL. Incurrent grid of a halfway inflated house. The somewhat thicker main fibrils are intersected by many considerably thinner transverse fibrils. Phase contrast micrograph. Scale 15 µ.

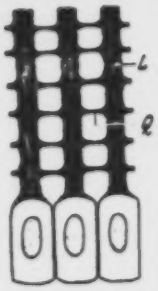

Fig. 41. Oikopleura dioica FOL. Greatly enlarged schematic representation showing the formation of the fibril network of the incurrent grid. At regular intervals, the longitudinal fibrils (L) produce laterally short transverse fibrils (Q) which fuse with each other.

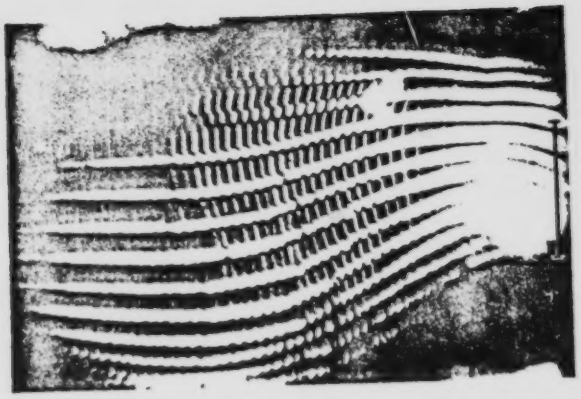

Fig. 42. Oikopleura albicans LEUCKART. Incurrent grid of a halfway inflated house. Micrograph taken between two crossed Nicols. Scale 50 µ. Compare with Fig. 40.

The intracellular processes are the same as in the other cells, only the secretion mechanism is different from that of the normal oikoplasts. Like FOL's oikoplasts, the giant cells secrete a gelatinous cushion which serves as a stable support for the fibrils to be produced and which disappears later (Fig. 38). Its boundary membranes are left behind and form the covering membranes for the incurrent orifices (Fig. 39a and b). The "microcells" are responsible for producing the grids (Fig. 40).

Very minute main fibrils are secreted by the "microcells" perpendicular to their outer cell boundary. The fibril thickness is approximately equal to the cell diameter. Two short adjoining, extremely delicate, transverse fibrils fuse with each other at certain regular intervals (Fig. 41). These transverse fibrils develop in the same manner as the secondary fibrils of the filter sytem.

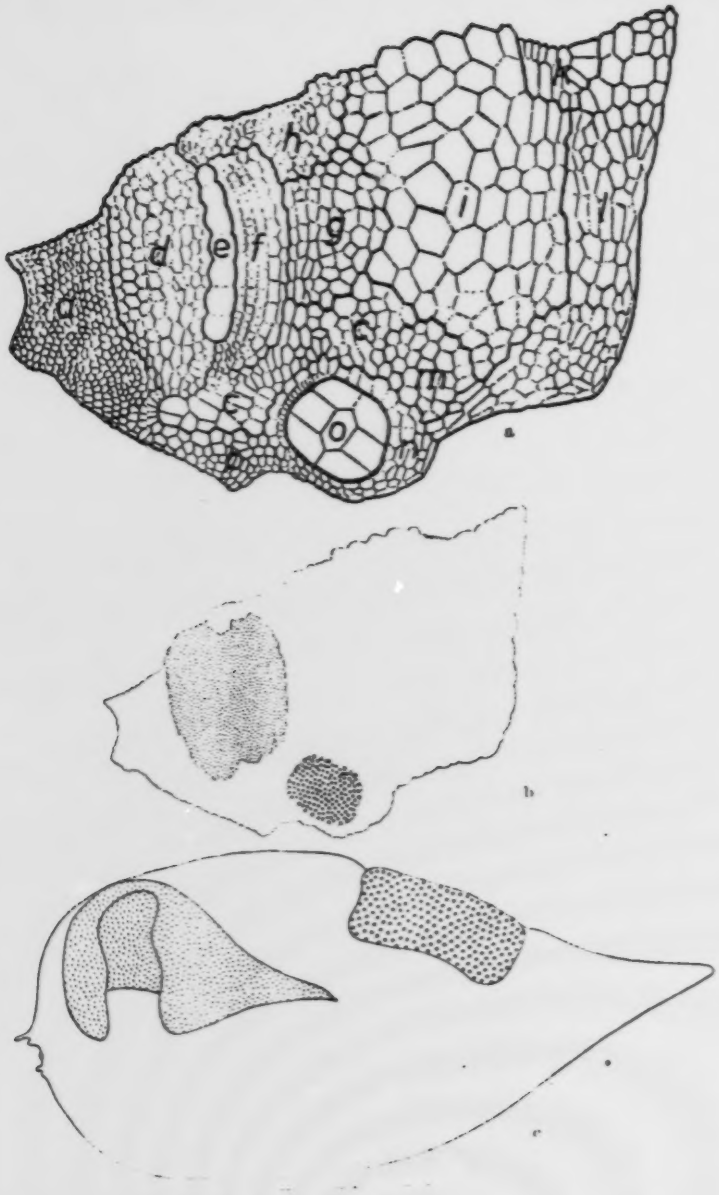

Fig. 43a–c. Oikopleura dioica FOL. Schematic representation of the relationships between the individual oikoplast groups and the completed house.

a Division of the oikoplastic epithelium into "BUCKMANN's regions". Lettering as in Fig. 2.

b FOL's and EISEN's oikoplasts outlined on the oikoplastic epithelium.

c Feeding apparatus and incurrent grid produced by the secretory activity of FOL's and EISEN's oikoplasts.

The fibrils of the incurrent grids in Oikopleura dioica FOL are so extremely delicate that their fine structure cannot be discerned. This is unlike Oikopleura albicans LEUCKART, where the string of secretory granules is still visible in the fibrils (Fig. 42). In any event, a screen with extremely minute apertures is produced in this manner. p. 35

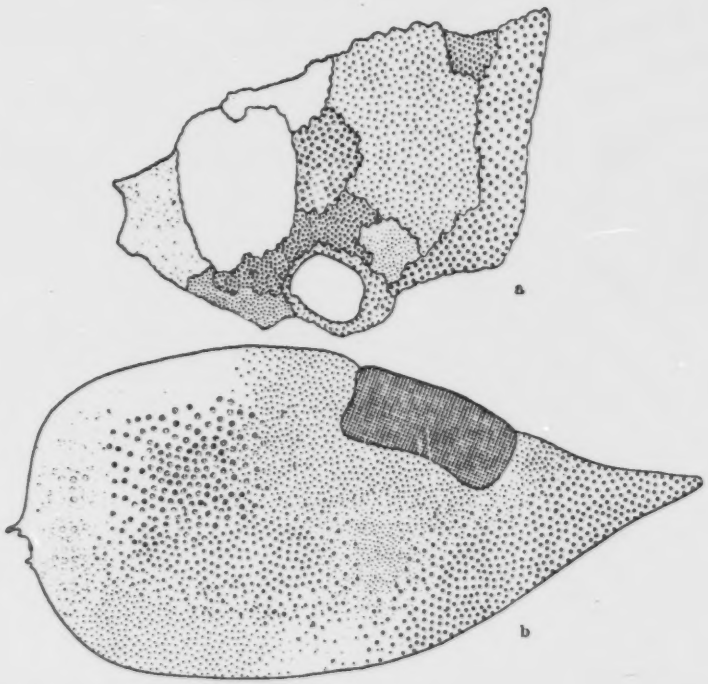

Fig. 44a and b. a. Outline of the remaining cell groups on the oikoplastic epithelium.
b. Secretory activity of the individual cell groups to produce the complete house. Corresponding parts appear in the same pattern. Oikoplastic epithelium and house have been represented at different scales and not at the natural ratio of about 1:100 (oikoplastic epithelium: inflated house). The same applies to Fig. 43.

The number of main fibrils in the house which is not yet fully inflated corresponds in Oikopleura dioica FOL to the number of "microcells", while the number of transverse fibrils fluctuates beween 100 and 120. These fluctuations can be attributed to the different ages of the animals, i.e. older animals produce more transverse fibrils than younger ones. The situation is similar as far as the number of primary and secondary fibrils of the feeding apparatus is concerned. The square apertures of the incurrent grids in the house which is not yet fully inflated have a mesh size of 1 µ. It is evident that only the most minute planktonic organisms and suspended particles can penetrate this grid.

The secretory activity of the cells produces complex houses and feeding apparatuses. Glandular secretion in Oikopleura dioica FOL is completely synchronous, with each individual cell supplying the secretion for a particular part of the house. When we compare the various stages of house formation and inflation with the BÜCKMANN regions which were selected arbitrarily at first, we will note that certain cell groups are associated with certain parts of the house. Fig. 43a–c illustrates these relationships between FOL's oikoplasts and the feeding apparatus and between EISEN's oikoplasts and the incurrent grids. I found similar relationships when I compared other cell groups with houses at different stages of inflation. The presence of "exuvial bodies" provided valuable help in orientation. Although it is not possible to pinpoint the exact cells involved, we nonetheless know approximately which cell groups are responsible for secreting a certain part of the house. These relationships are illustrated in Fig. 44a and b. p. 36

VIII. Chemical Composition of the House

All attempts at finding out more details about the chemical composition of the house have failed so far because previous investigators assumed that the tunics or houses of all tunicates were composed of cellulose. None of the investigators has so far been successful in finding cellulose in appendicularians by means of tests normally used for plants. FOL (1872) found already that neither zinc chloroiodide solution nor iodine-sulfuric acid produced any reaction, while the same tests carried out on ascidians were positive and showed the presence of cellulose. Chemical analysis and roentgenographic studies also show a relatively high percentage of cellulose (F.E. SCHULZE 1863, O. HERTWIG 1873, SUTRA 1932) which is identical with plant cellulose. More recent studies, however, tend to point to a chitinous mass as the ground substance in the ascidians (BRIEN 1948).

Analytical findings of this nature are not yet available for the appendicularians since considerable difficulties are involved in obtaining enough houses for analysis. Similarly, studies of the general chemical behaviour of the ground substance have not yet been undertaken and we, therefore, have no clues whatsoever as to what we can expect. Accordingly, all we can do is to use microchemical tests to get some idea about the chemical composition of the house. As previously mentioned, the substance in question is apparently uniform and stains with almost all stains, but this is not sufficient to make any definite statements.

Of the mineral acids, only concentrated sulfuric acid visibly affects the house and dissolves it completely. Neither concentrated nor dilute hydrochloric acid and nitric acid produce any noticeable change even after prolonged boiling. p. 37

Boiling the house substance in concentrated alkali for about 1/2 hr. also dissolves the house.

The fact that the substance dissolves completely in concentrated sulfuric acid indicates the presence of a carbohydrate compound, i.e. a polysaccharide, since dissolution is accompanied by typical carbonization and the solution has subsequently a slightly yellow hue. On the other hand, solubility in concentrated alkali indicates an affinity with chromotropic substances as they occur mainly in tissues of embryos and in cartilages (HOLMGREN 1940) and are characterized by the presence of sulfuric acid esters with a high molecular weight.

We can conclude from the above that the appendicularian house does not consist of chitin[1] since it reveals none of the typically chitinous behaviour relative to acids and bases. - Chitin is insoluble in boiling alkalis, yet soluble in hot concentrated hydrochloric acid, sulfuric acid and concentrated anhydrous formic acid, none of which applies to the house of the appendicularians. - Similarly, the microchemical tests for chitin (chitosan reaction, zinc chloroiodide test, α and β naphthol reaction) are negative. All typical tests for cellulose are likewise negative.

---

[1] BRIEN (1948) has recently referred to the substance of the appendicularian house as being chitinous.

However, the Hotchkiss reaction[2], BERG's ninhydrin reaction and metachromatic staining with α toluidine blue according to LISON and according to HEMPELMANN (1950) produced some results.

The Hotchkiss reaction stains all structures which contain polysaccharide a bright red. This colour reaction was observed simultaneously on the house wall, the outer cell boundary of the oikoplasts and the granules which lie on the inner side of the cell boundaries facing the interior of the body (Fig. 22).

The ninhydrin reaction stains low protein substances which are interlinked with the polysaccharide. For this purpose, fresh material fixed in Formol was teased out and boiled in a 0.1% ninhydrin solution for 2 min. Although the specimens stained only a weak blue, the blue coloration appeared wherever the polysaccharide was present in the form of strands, except for the polysaccharide granules which formed the prosecretion. The nucleoli were surrounded by a more brightly stained zone. As a result, the formation of amino groups may be attributed to them. p. 38

Polysaccharide and amino groups are somehow interlinked and together make up the substance of the house.

---

[2]The modified Hotchkiss reaction:
1. Hydrolysis in periodic acid, 2 hrs.
2. Rinse in 70% alcohol, for a short time.
3. Potassium iodide/sodium thiosulfate solution, 5 min.
4. Rinse in 70% alcohol for a short time.
5. Fuchsin/sulfuric acid, 45 min.
6. Continue as in the Feulgen reaction.

Preparation of the solutions:
Periodic acid solution: 235 mg of $NaIO_4 \cdot 3H_2O$, 17.5 cm^3 of n/10 CH_3COOH, 25 cm^3 of distilled water.
Potassium iodide/sodium thiosulfate solution: 1 g of KI, 1g of $Na_2S_2O_3 \cdot 5H_2O$, 19.5 cm^3 of distilled water, 30 cm^3 of absol. alcohol, 1 cm^3 of n-HCl.

LISON's[1] α toluidine blue stains the secretion and the house metachromatically violet. Although toluidine blue stains many acid substances, metachromatic staining is specific to chromotropic substances which are characterized by the presence of sulfuric acid esters with a high molecular weight, such as chondroitin sulfuric acid or mucoitin sulfuric acid (GLICK 1948). HEMPELMANN (1940) reports that chondroitin and mucoitin can be distinguished histologically with toluidine blue. When using his dilution of 1:280,000 in 18% alcohol, toluidine blue stained the substance of the house and the secretory strands a metachromatic violet after 30 min. This finding clearly indicates the presence of chondroitin. HEMPELMANN (1940) reports that the mucoitin complexes on the other hand do not stain at all with toluidine blue.

It follows that chondroitin is present in the substance of the appendicularian house. This finding is based solely on the above-mentioned microchemical reactions and may be viewed as a starting point for later analytical studies. The histochemical tests show in any event that the appendicularian house does not contain any cellulose. Rather, the house is composed of a polysaccharide in conjunction with NH_2 groups, i.e. an esterified amino sugar.

Similar exoskeletal substances are found in arthropods, molluscs, and bryozoans, and chondroitin sulfuric acid is present in the cartilage of vertebrates.

---

[1] α toluidine blue according to LISON: 0.1% solution in 1% alcohol.

SCHÄFER (1871) reported that an analysis of the pyrosome tunic yielded the following results:

water	about 95%
cellulose	about 1.1%
nitrogenous substances	about 3.2%
ash	about 0.7%.

When we based these results on dry matter, rather than the weight of fresh substance as SCHÄFER did, we obtain values which shed a completely different light on the composition of the pyrosome tunic, i.e. it contains 62.4% nitrogenous substance, in addition to 13.9% ash and 23.7% cellulose. Taking this finding in conjunction with the report by NEUMANN [1935 (1)], regarding successful identification of chondrin in a complex procedure, we may assume that the main constituent of the house in the appendicularians (which are related to the pyrosomes) is a similar substance. It may well be that this substance is identical in appendicularians and pyrosomes and that we can perhaps establish new phylogenetic relationships among the tunicates based on this finding. p. 39

IX. Development of the "Exuvial Bodies"

Earlier studies of the formation of the appendicularian house did not suggest that cells of any kind were embedded in the gelatinous mass. No attention was, therefore, given to the fact that cells were discharged from the oikoplastic epithelium complex. Only SEELIGER [1893 (2)] reported that he found free cells or parts of the cells in the "outer mucous layer" (which can, of course, only mean the house or its rudiment)

in specimens of all ages of Oikopleura cophocerca GEGENBAUR and that these cells originated from the ectodermal main epithelium. He stated that only a few of these cells were present, i.e. 16-18 at the most, and that they were always near the epithelium.

SEELIGER [1893 (2)] continued as follows:

> "Since these cells do not persist but are transitory and are found in specimens of all ages, although few in numbers, the total number of ectodermal elements discharged by the animal is, presumably, quite considerable. Since these discharged elements are histologically completely identical with ectodermal cells, there is no doubt that these elements are cells from the ectoderm. The absence of free mesodermal cells in the body cavity eliminates the possibility that the same process might take place as in the ascidians and salps..."

LEUCKART (1854) also mentions granular inclusions in the appendicularian house which are either grouped side by side or in smaller or larger clusters.

LOHMANN (1933), on the other hand, rejects the assumption that cells are discharged from the oikoplastic epithelium. If his view were correct, there would be cell constancy, as postulated by MARTINI [1901 (1), 1910] for appendicularians. LOHMANN (1931, 1933) assumed that the cell SEELIGER [1893 (2)] observed had been torn from the cell complex as a result of preparation or as a result of pathological changes. Presumably, he himself never saw illustrations of such cells.

I would like to add already at this point that pathological changes of that nature will certainly not occur with such regularity. p. 40

In Oikopleura dioica FOL, cells were observed to break away regularly from the oikoplastic epithelium (Fig. 45). The following questions arose in conjunction with this process:

1. How do these cells break away?
2. Are the discharged cells replaced and how does the replacement, if any, occur?
3. What are the chemical properties of these cells compared with those in the oikoplastic epithelium?
4. For what purpose are these cells discharged?
5. Once we have answered the above questions, can we still talk about cell constancy in the appendicularians sensu MARTINI [1909 (1) and 1910]?

A. Mechanism of Cell Discharge

The cells are always discharged at the same location at regular time intervals. This finding alone indicates that the discharge is not purely accidental and concerns old cells. On the contrary, it is a well-ordered process.

The centres of cell discharge in Oikopleura dioica FOL are MARTINI's field, the oblique line, the dorsal cells, the mouth region and the posterior rosette (Figs. 3 and 45). These regions coincide almost

precisely with the location of the "exuvial bodies" described by LOHMANN (1896) for Oikopleura dioica FOL. They are, in fact, identical with them, as my investigations have shown.

LOHMANN (1896) gives the following description of the "exuvial bodies":

> "Known only from the labradoriensis group are strange structures resting on the house rudiments. I have called these structures "exuvial bodies" because I assume that they play a very important role during inflation of the house which is equivalent to a moulting process in other cuticle-bearing animals... Although the formation of the exuvial bodies starts, of course, from the oikoplastic epithelium, and I have been able to determine in one instance that the principles according to which they are arranged are dependent upon certain cell sequences, I have been unable to establish any particular relationship between the oikoplast structure on the lateral surface of the body and the formation of the exuvial bodies. Similarly, no relationships have been found to exist with other structures."

The relationships between the "exuvial bodies" and the oikoplastic epithelium can only be clearly understood when we apply the Feulgen reaction. The "exuvial bodies" are Feulgen-positive and we can, therefore, trace their entire development. The process is as follows:

When the secretion of a house rudiment has just been completed, cells are discharged from certain locations on the epithelial complex p. 41

(see Figs. 45 and 46). Yet cytological changes already occur in these cells while the house is being secreted.

While the nuclei in the normal, functioning adjoining cells resemble a reticulated sphere and lie within a diffuse, very finely granulated cytoplasm, the cells to be discharged show signs of necrobiosis. Their nuclei lose their fine structure and gradually become completely compact (pycnotic) while the cytoplasm becomes fibrillar. As the process of necrobiosis continues, the numerous nucleoli disappear together with the heterochromatic nuclear constituents (Fig. 47). As long as the cell remains within the epithelial complex, its size remains practically unchanged, although the nucleus may slightly change in size.

In the subsequent stage, the altered cell is virtually expelled from the oikoplastic epithelium. At the same time, one of the adjoining cells expands and begins to close the gap, while the cell which has lost its function of secreting the house is pushed on top of it (Fig. 48). When the cell is discharged, the house rudiment which rests on the epithelium lifts from the discharge point. As soon as the cell lies outside of the epithelial complex, it undergoes further changes. The nucleus shrinks to about 2/3 of its size, and both cytoplasm and cell membrane dissolve gradually. p. 42

After this process is completed, nothing but a shrunken nucleus is left under the house wall. Its appearance does no longer identify it as a nucleus and its origin can only be explained on the basis of the previously described process. Like all nuclei, it contains a large amount of Feulgen-positive substance which can be identified without difficulties.

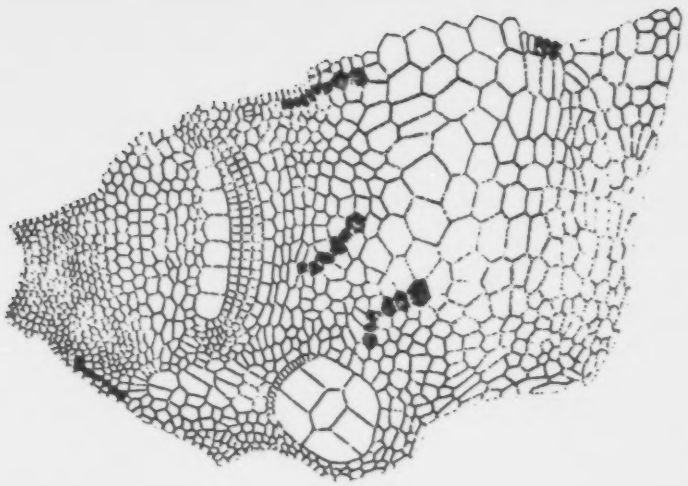

Fig. 45. Oikopleura dioica FOL. Oikoplastic epithelium showing the centres where exuvial bodies are formed.

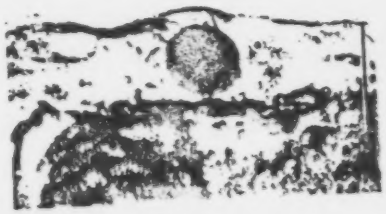

Fig. 46. "Exuvial body" located between two house rudiments. Only the nucleus is still visible. Bouin-Allen, Feulgen. Micrograph. Scale 10 µ.

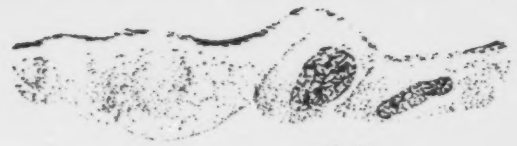

Abb. 47.

Abb. 48.

Fig. 47. Oikopleura dioica FOL. Three cells ready to emerge from the oikoplastic epithelium. The cells are completely compact and the cytoplasm has a fibrillar structure. The nucleoli can be discerned in the adjoining nuclei. The house rudiment has already been secreted.

Fig. 48. Separation of the house rudiment from the oikoplastic epithelium following the discharge of a cell. The adjoining cell closes the resulting gap. Scale 10 µ.

Abb. 49.

Abb. 50.

Fig. 49. Two "exuvial bodies" are shown under the first house rudiment which has not yet expanded; a cell which is ready to emerge from the epithelium lies under the second house rudiment.

Fig. 50. Two "exuvial bodies" between the two house rudiments. (The nucleus has not been shown in one of the cells.) Beneath the second house rudiment, a cell is shown emerging from the oikoplastic epithelium. Scale 10 µ.

If a new house is secreted before inflation of the first house, we will find the above-mentioned cell remnants enclosed beween the inner boundary membrane of the first house rudiment and the outer boundary membrane of the second house rudiment. The new "exuvial body" is then discharged beneath the second house, while the first "exuvial body" remains superimposed on it (Figs. 49 and 50).

Therefore, if three house rudiments were present, we would find "exuvial bodies" lying one on top of the other at about the same location, while a new exuvial body is being formed underneath[1].

The discharge of an "exuvial body" after secretion of a house rudiment is immediately followed by the production of a new house; once this new house has been secreted, an "exuvial body" is formed at the same spot as in the preceding process. p. 43

B. Problem of Cell Restitution

To ensure that the oikoplastic epithelium continues to secrete a perfect house and a properly functioning feeding apparatus requires replacement of the cells which are discharged with each formation of a house. Since about 60 cells are discharged during each process (this number is not constant and fluctuates within narrow margins), each animal loses 60 cells many times over during its life span. It should also be noted

---

[1]The presence of three house rudiments was observed once at the beginning of our investigations, but no importance was then attached to the "exuvial bodies" because we were unaware of the process involved.

that each animal sheds its house 5-6 times over a 24 hour period and has a life span of about six months, as suggested by SEELIGER (1893) based on the cyclic appearance of mature animals. It must be assumed that these cells will be replaced once they have been discharged because a reduction of the number of cells in the oikoplastic epithelium would inevitably result in serious defects during house production. The animal would perish under such circumstances.

According to our present findings, the oikoplasts in Oikopleura dioica FOL are polyploid and do not undergo normal mitosis. Replacement of the discharged cells could, therefore, only occur through endomitosis with subsequent cell constriction and separation. Occasionally, the oikoplastic epithelium contained nuclei with the heterochromatin shifted towards the two nuclear poles and a constriction along the equator. These nuclei were found in the posterior rosette. However, I was unable to find out more about their behaviour. This problem requires a special study which will be conducted at a later date. Similarly, I was unable to determine whether the cell divisions associated with the discharge of the "exuvial bodies" originated always from the same cell. All these investigations failed because culturing of Oikopleura dioica FOL requires a very long time in captivity. p. 44

C. Chemical Properties

Since the "exuvial bodies" are dead nuclei or parts of nuclei, their thymonucleic acid can still be detected by means of the Feulgen reaction. However, neither nucleoli nor protein substances can be detected in these nuclei. The cytoplasmic remnants, which are

initially still present, were not found to contain any secretory mass. Neither basic nor acid stains stained the cytoplasmic remnants clearly and distinctly since they absorb very little of the stain.

The conclusion we can draw from this is that once the discharged cells have elaborated the substance for, presumably, a single house, i.e. during one secretion phase, they are subsequently no longer useful for any secretory activity. It may well be that further secretory activity was not intended anyway. Their functions in this respect are now exhausted and, after being discharged from the epithelium, they only have one single new function.

D. Function of the "Exuvial Bodies"

The cell walls of the discharged cells dissolve and the cytoplasm presumably liquefies through enzymatic action, while the nucleus becomes compact and thereby remains preserved. This liquid substance presumably spreads between the oikoplastic epithelium and the house rudiment and acts perhaps as a kind of lubricant during separation of the house rudiment from the epithelium. It may assist in inflating the house uniformly and smoothly. The function of the "exuvial bodies" as such is not separation, as suggested by LOHMANN (1933). In my view, they can only be regarded as the end products and the remnants of cells which were responsible for the formation of the separating substance. They merely mark, as it were, the locations from which the separating substances emerged. p. 45

More or less comparable processes are known only from the moulting of lepidopteran larvae to the pupal stage (Ephestia kühniella Z.; KÜHN and PIEPHO 1938). I would not compare this directly with the formation of the appendicularian house, but at least indicate the existing analogy:

> The large glandular cell of the Versonian gland in the larvae of Ephestia kühniella Z. is evacuated during the moulting to the pupa. Its aqueous secretion, together with the fluid which apparently originates from the hypodermis and is present between the hypodermis and the cuticula of the caterpillar long before the secretion of the Versonian gland, constitute the lubricant which is known as exuvial fluid. This fluid plays an important role during shedding of the remaining upper boundary layer of the caterpillar cuticula, i.e., during the moult to the pupa. Hormones are said to be certainly responsible for this process.

It is as yet unknown which substances are responsible for the exuviation process in Oikopleura dioica FOL, and all I can do is point to the analogy of the moulting process in lepidopteran larvae without being able to interpret it.

Since the exuvial bodies always emerge at the same location, it may be assumed that a separation-promoting secretion is formed here. Also, the centres where the exuvial bodies are formed (see Fig. 45) are distributed so uniformly over the entire oikoplastic epithelium that a

uniform distribution of the "exuvial fluid" over the epithelial surface and within the required time is ensured. If one of the centres where the exuvial bodies are formed were to fail, malformations would ensue, and this is particularly worth paying attention to in the future.

E. Question of Cell Constancy

MARTINI (1910) reported that the number of oikoplasts in Fritillaria pellucida BUSCH was always 446. He, therefore, postulated cell constancy for the oikoplastic epithelium and assumed that all cells would be preserved during the life span of the animal and that no cells would be replaced.[1] Although the formation of "exuvial bodies" has so far only been known from the oikopleurids of the labradoriensis group, it may well be that still other cells, for example, in the intestinal tract, are gradually being replaced during the relatively long life of the appendicularians. For example, I found mitoses in the cells of the intestine even in adult animals. p. 46

Perhaps cell constancy in the strict sense does not apply to appendicularians. At most, we might talk about numerical constancy of the cells in the oikoplastic epithelium even though the number of cells fluctuates within certain narrow limits.

---

[1] In addition, MARTINI [1909 (1)] noted that all histological elements in Oikopleura longicauda VOGT were constant. Since that time, the appendicularians have been regarded as cell-constant animals. Further studies will have to show whether this classification is justified.

F. Definition of "Exuvial Bodies"

Based on the present findings, I propose the following new definition for the "exuvial bodies" of the appendicularians:

"Exuvial bodies" sensu LOHMANN are the nuclei of the cells which emerge from certain locations on the oikoplastic epithelium after the secretion of each house and whose cytoplasm is presumably converted into a separating substance between the house rudiment and the oikoplastic epithelium.

X. Affinities of the Appendicularians with the Other Tunicates Based on the Present Findings

A character common to all tunicates is the secretion of the tunic or the house from a simple epithelium. While this epithelium is restricted to a certain region in the appendicularians, it covers the entire body surface in representatives of the other classes.

A. Formation of House and Tunic in the Tunicates

The tunicate house performs various functions in the various tunicate groups:

At rhythmic intervals the pelagic appendicularians secrete a delicate thin layer which inflates to form the house and is again shed after a few hours. After the secretion of each house, cells emerge from the oikoplastic epithelium at certain locations and remain between the

epithelium and the house rudiment. When the house expands, these cells presumably act as "exuvial bodies" during separation of the house rudiment from the epithelium. In any event, cellular elements of the ectoderm are discharged into the house rudiment.

The ascidian tunic covers the main epithelium as a more or less thick and compact layer and forms the outer sheath around the body throughout the life of the ascidian. The thickness of the tunic increases to a certain limit in the course of its growth and the tunic incorporates many cellular elements not only of mesodermal, but also ectodermal, origin (JULIN 1892, SALENSKY, 1895).

The main epithelium of the Cyclomyaria also secretes a structureless (?) cuticular layer which, according to NEUMANN [1935 (2)], is the thinnest among all the tunicates. Cellular elements are additionally embedded in the tunic of Doliopsis (NEUMANN 1935). ULJANIN (1881, 1882) repeatedly observed live specimens of Doliolum mülleri KROHN shedding their tunic several times like the appendicularians. Shedding and new formation of a tunic is no doubt very limited in Doliolum, yet it is obviously a behaviour which it has in common with the appendicularians. The tunic of Doliolum mülleri KROHN does not contain cellular elements [NEUMANN 1935 (2)], but so far we also know "exuvial bodies" in appendicularians only from representatives of the labradoriensis group. p. 47

We see, therefore, that in the Ascidiaceae, the Pyrosomida and the Desmomyaria, cellular constituents migrate unrestricted into the tunic substance regardless of whether they are of mesodermal or of ectodermal

origin. The presence of cells in the tunic substance is limited to a few species in the Cyclomyaria and the Appendiculariae. All orders of the tunicates, however, have the character in common that in most of their representatives cells migrate into the tunic substance.

B. Chemical Comparison of the Main Constituents of the Tunic Substance

Cellulose was found to be the ground substance in the Ascidiaceae (PAYEN 1846; SCHÄFER 1871; SAINT-HILAIRE 1931; GRASSMANN, ZECHMEISTER, TOTH and STADLER 1933) and in the Desmomyaria (LÖWIG and KÖLLIKER 1846). The ground substance of the pyrosome tunic (SCHÄFER 1871) and of the appendicularian house consists mainly of nitrogenous substances. The test for cellulose was also negative in the Cyclomyaria [NEUMANN 1935 (2), ULJANIN 1882] and we have to assume that their ground substance is also nitrogenous in nature. In addition, chondrin has been found in the tunic of the pyrosomes [NEUMANN 1935 (1)] and we can, therefore, assume that the tunic substance is the same in appendicularians and pyrosomes.

Table 1 provides an overview of the various properties of the substance which makes up the house or the tunic.

The greatest similarities are between the Cyclomyaria and the Appendiculariae as far as the above-mentioned properties are concerned. Although we do not yet know the precise composition of the ground substance of the tunic, the fact alone that cellulose is not present suggests a substance similar to chondrin, perhaps even the same

p. 48

substance as in the pyrosomes and appendicularians. Migration of mesodermal cells into the tunic has not yet been observed in the Cyclomyaria. Yet there is the distinct possibility that the cells found in the tunic substance are, after all, of ectodermal origin in view of the fact that Cyclomyaria can replace the tunic. As obvious as these conclusions might be, they still require comprehensive substantiation.

Summary

1. The present study was undertaken to investigate the formation of the appendicularian house in all its stages in order to obtain a complete overview of the processes involved. The studies were conducted on specimens of Oikopleura dioica FOL from the mud-flats of the German Bight. Oikoyleura dioica FOL is probably a geographical variant of the nominate form which has been described from the Mediterranean.

Table 1

Subclass or order	Ground substance of the tunic or the house	Ability to replace the tunic or house	Cellular elements in the substance of the tunic or house	Origin of the cellular elements in the tunic or house
Ascidiaceae	cellulose	lacking	continuous migration of cells	mesoderm and ectoderm to some extent
Desmomyaria	cellulose	lacking	continuous migration of cells	mesoderm, as far as is known to date
Pyrosomida	nitrogenous substance, chondrin	lacking	continuous migration of cells	mesoderm, as far as is known to date
Cyclomyaria	nitogenous substance, no cellulose	present	few cells; not present in all species	origin unknown
Appendiculariae	nitrogenous substance, chondrin	present	few cells; not present in all species	ectoderm

2. A brief description outlines what has previously been known about the formation of the appendicularian house. While the tunic in the other tunicates covers the entire body surface, the formation of the house is restricted in the appendicularians to the oikoplastic epithelium.

p. 49

3. The material has been briefly described to indicate the differences which exist between the nominate form from the Mediterranean and the present species with respect to the arrangement of the giant cells of FOL's oikoplasts and the intestinal tract.

4. Unlike other species, Oikopleura dioica FOL does not have branched nuclei in the oikoplastic epithelium. All cells of the oikoplastic epithelium are presumably tetraploid (two heterochromatic elements); but the level of polyploidy may even be much higher. Mitoses were not observed in the oikoplastic epithelium, but merely signs of cell constrictions. Endomitotic processes were not detected.

5. Chromosomes were studied in diploid tissue cells of larvae, in spermatocytes and oocytes. It is assumed that Oikopleura dioica FOL is haploid and has three telocentric chromosomes, one of which is somewhat longer, while the two others are essentially the same length.

6. The number of nucleoli in the variously sized oikoplast nuclei ranges from 3 to 50 and increases with the size of the cells.

7. The secretion processes in the oikoplasts take place in three

separate, parallel phases. Elaboration of prosecretions and of final secretions and the formation of a new house rudiment occur side by side.

8. Histochemical methods were used to identify chondrin as the substance which is later secreted to form the house. It is composed of polysaccharides, which are ingested as nutrients, and of protein substances which are formed presumably by the nucleoli. Only pure polysaccharide, but no protein, is present in the precursors of the secretion.

9. The final secretion takes the form of moniliform strands which lie within the cell but are not in direct contact with the nucleus.

10. The nucleoli are transformed during the secretion process; they increase in size and eventually become ribbon-shaped at the end of the process.

11. The house is formed in the following manner: The secretory strands are appressed from the inside against the outer cell boundary and are separated from the cell body by a fine membrane. This structure separates from the cell and fuses with the secretion products of the other oikoplasts. Initially, seams outline the margins where the individual secretion products have fused. These seams on the house rudiment mirror the polygonal tesselation of the oikoplastic epithelium, but disappear as the house expands.

12. Unlike other authors, I found that the wall of the house was not composed of fibrils. It would appear that the house becomes inflated by the incorporation of water molecules, i.e. as a result of a specific swelling process. p. 50

13. The feeding apparatus is secreted essentially in the same manner as the house. However, some differences are noted. The three longitudinal rows of 27 cells each, which are disposed along FOL's giant cells, secrete a fibril system that consists of three types of fibrils, namely main, primary, and secondary fibrils. The close interaction between the individual cells has been particularly emphasized.

14. The incurrent grids are secreted by the seventeen small cells of EISEN's oikoplasts. Seventeen longitudinal fibrils and 100-120 transverse fibrils are formed. Here as well, the secretion mechanism has undergone specialization to produce the particular structure of the fibril system. The square apertures of the incurrent grids have a mesh size of 1 μ in a house which has not completely expanded.

15. Various chemicals were used to analyze the house substance. Its behaviour differs from that of chitin. It exhibits the properties of chromotropic substances when stained with toluidine blue.

16. Up to 60 "exuvial bodies" are released during each exuviation. These "exuvial bodies" are the pycnotic nuclei of cells discharged from the oikoplastic epithelium. Their cytoplasm dissolves after discharge and is believed to form an exuvial liquid.

17. The manner in which the discharged cells are replaced in the oikoplastic epithelium has not been completely resolved. Replacement of these cells is, however, necessary since subsequently formed houses would be flawed otherwise.

18. Contrary to reports of earlier investigators, cell constancy in the strict sense (see 16. and 17.) does not exist in appendicularians.

19. The structure and function of the feeding apparatus with its filter system has been described by way of drawings. Reference has been made to the close interrelationship between food intake and house formation which form a closed physiological system.

20. The properties of the tunics and houses of all tunicates have been compiled to discuss the affinities between the representatives of the various groups. Both the appendicularian house and the pyrosome tunic are composed of chondrin.

Literature

Literatur.

(1) [illegible] J. Coll. Sci. Tokyo 23, 1 (1907). (2) [illegible] Mitt. zool. Stat. Neapel 9, 475 (1889). (3) [illegible] Phosphorismus gefärbter anisotroper Substanzen des Tierkörpers. Arch. f. Physiol. 24, 301 (1889). (4) Arnold, J.: Über Bau und Sekretion der Drüsen der Froschhaut. Arch. mikrosk. Anat. 65, 49 (1905). — (5) Bauer, H.: Mikroskopisch-chemischer Nachweis von Glykogen und einigen anderen Polysacchariden. Z. mikrosk.-anat. Forschg 33, 143 (1933). (6) Becker, W. A.: Über einige Streitfragen der Zellteilung. Z. Zellforschg 23, 253 (1935). — (7) Berg, W.: Über die Anwendung der Ninhydrinreaktion auf mikroskopische Präparate zum Nachweis niederer Eiweißkörper. Pflügers Arch. 195, 541 (1922). (8) Bowen, R. H.: Cytology of gland secretion. Quart. Rev. Biol. 4, 484 (1929). (9) Brien, P.: Tuniciers. Traité Zool. 12 (1948). (10) Bückmann, A.: (1) Bemerkungen über Appendicularien aus der Ausbeute der deutschen Südpolar-Expedition. Zool. Anz. 59, 205 (1923). (11) (2) Beitrag zur Kenntnis der Appendicularien auf Grund der Ausbeute der deutschen Südpolar-Expedition. Diss. Leipzig 1923. (12) Copelata. Tierwelt der Nord- und Ostsee 12, 1 (1926). (13) Tunicata. Tierwelt Deutschlands 17, 143 (1930). (14) Appendicularia I—III. Cons. int. Expl. Mer 7, 2 (1945). — (15) Caspersson, T.: Über den chemischen Aufbau der Strukturen des Zellkernes. Skand. Arch. Physiol. (Berl. u. Lpz.) 73, Suppl. 8, 91 (1936). (16) Damas, D.: Contribution a l'étude des Tuniciers. Arch. Biol. 20, 747 (1904). (17) Delsmann, H. C.: Weitere Beobachtungen über die Entwicklung von *Oikopleura dioica*. Tijdschr. nederl. dierkd. Ver. ser. (2) 12, 199 (1912). (18) Der Ursprung der Vertebraten. Zool. Anz. 41, 175 (1913). (19) Duthie, E. S.: The mechanism of glandular secretion. Arch. exper. Zellforschg 15, 325 (1934). (20) Essenberg, Chr. E.: Copelata from San Diego Region. Univ. California Publ. Zool. 28, 399 (1926). (21) Fol, A.: Note sur la régénération de la tunique chez les Tuniciers. Bull. Soc. zool. France 33, 79 (1908). (22) Fol, H.: Études sur les Appendiculaires du Détroit de Messine. Mem. Soc. Phys. Genève, 21, 445 (1872). (23) Frey, A.: Die submikroskopische Struktur der Zellmembranen. Jb. Bot. 65, 195 (1926). (24) Das Wesen der Chlorzinkjod-Reaktion. Jb. Bot. 67, 597 (1927). (25) Frey-Wissling, A.: Submikroskopische Morphologie des Protoplasmas und seiner Derivate. Protoplasma Monographie, 15. Berlin: Gebrüder Borntträger 1938. (26) Tunicin im Elektronenmikroskop. Protoplasma (Berl.) 39, 656 (1950). (27) Garstang, W.: The morphology of the Tunicata, its bearings on the Phylogeny of the Chordata. Quart. J. Microsc. Sci., N. S. 72, 51 (1928). (28) On the dextricolic condition in Tunicates. Proc. Leeds. Philos. Lit. Soc., Sci. Sect. 1, 506 (1929). (29) On a new Appendicularian, Bathochordeus sp. from Bermuda, with a revision of the genus. Proc. Linnean Soc. London 36, 131 (1935). (30) Garstang, W., and E. Georgson: Copelata. Rep. Brit. Antarct. („Terra Nova"). Exped. Zool. 8, 263 (1935). (31) Geitler, L.: Grundriß der Cytologie. Berlin: Gebrüder Borntträger 1934. (32) Wachstum des Zellkernes in tierischen und pflanzlichen Geweben. Erg. Biol. 18, 1 (1941). (33) Gersch, M.: Untersuchungen über die Bedeutung der Nukleolen im Zellkern. Z. Zellforschg 30, 481 (1940). (34) Glick, D.: Techniques of histo- and cytochemistry. New York: Interscience publishers Inc. 1949. (35) Grassmann, W., L. Zechmeister, L. Toth u. R. Stadtler: Über den enzymatischen Abbau der Zellulose und ihrer Spaltprodukte. Liebigs Ann. 503, 167 (1933). (36) Grobben, K., u. A. Kühn: Lehrbuch der Zoologie. 4. Aufl. Berlin: Springer 1932. (37) Hammarsten, E., G. Hammarsten u. T. Torelli: Versuche über mikrochemische Reaktionen. Acta med. scand. (Stockh.) 68, 219 (1928). (38) Hempelmann, L. H. J.: Staining reations of mucoproteins. Anat. Rec., 78, 197 (1940). (39) Herdmann, W.: Revised classification of Tunicata. J. Linnean Soc. London 23, 558 (1891). (40) Hertwig, O.: Untersuchungen über den Bau und die Entwicklung des Zellulosemantels der Tunikaten. Jena. Z. Naturwiss. 7, 46 (1873). (41) Hirsch, G. Chr.: The theory of fields of restitution, with special reference to the phenomena of secretion. Biol. Rev. Cambridge Philos. Soc., 6, 88 (1931). (42) Das Problem des Wiedererratzes des Sekretstoffes in Drüsen. Riv. Biol. Perugia 24, 5 (1938). (43) Hirschler, J.: Plasmastrukturen in Tunikaten, Spongien und Protozoenzellen. Anat. Anz. 47,

289 (1914). (44) Holmgren, H.: Studien über Verbreitung und Bedeutung der chromotropen Substanzen. Z. mikrosk.-anat. Forschg 47, 489 (1940). (45) Huus, J.: *Ascidiaceae*. In Handbuch der Zoologie, Bd. 5, S. 401. Berlin 1937. (46) Ihle, J. E. W.: Bijdragen to de Kennis van de Morphologie en Systematik der Appendicularien. Acad. Proefschrift 1906. Universiteit Amsterdam. (47) Über den Endostyl und die systematische Stellung der Appendicularien. Zool. Anz. 31, 770 (1907). (48) Die Appendicularien der Siboga-Expedition. Siboga-Exp. 56c, 123 (1908). (49) Über die sogenannte metamere Segmentierung des Appendicularienschwanzes. Zool. Anz. 35, 404 (1910). (50) Die Appendicularien. Erg. Zool. 3, 463 (1913). (51) *Desmomyaria*. In Handbuch der Zoologie Bd. 5, S. 401. Berlin 1935. (52) Julin, Ch.: Recherches sur la phylogenèse des Tuniciers. Z. Zool. 78, 544 (1904). (53) Karrer, P.: Lehrbuch der organischen Chemie, 9. Aufl. Leipzig: Georg Thieme 1943. 1008 S. (54) Klaatsch, H.: Über Kernveränderungen im Ektoderm der Appendicularien bei der Gehäusebildung. Morph. Jb. 23, 142 (1895). (55) Korschelt, E.: Beiträge zur Morphologie und Physiologie des Zellkernes. Zool. Jb., Anat. 4, 1 (1891). (56) Vergleichende Entwicklungsgeschichte der Tiere. Tunikaten, S. 1010—1032. Jena 1936. (57) Kowalewsky, A.: Einige Beiträge zur Bildung des Mantels der Ascidien. Mém. Acad. Sci. St. Pétersbourg 38, 1 (1892). (58) Krüger, P.: Rolle des Kernes im Zellgeschehen. Naturwiss. Wschr. 14, 1022 (1926). (59) Kühn, A., u. H. Piepho: Die Reaktionen der Hypodermis und der Versonschen Drüsen auf das Verpuppungshormon bei *Ephestia kühniella* Z. Biol. Zbl. 58, 12 (1938). (60) Kunike, G.: Nachweis und Verbreitung organischer Skelettsubstanzen bei Tieren. Z. vergl. Physiol. 2, 233 (1925). (61) Lee, A. B.: Recherches sur l'oogenèse et la spermatogenèse chez les Appendiculaires. Recu. zool. Suisse 1, 645 (1884). (62) Lehnartz, E.: Chemische Physiologie, 8. Aufl. Heidelberg: Springer 1948. 476 S. (63) Leuckart, R.: Zoologische Untersuchungen. *Appendicularia albicans*. 2 (1854). (64) Lison, L.: Histochimie animale. Paris: Gauthier-Villars 1936. 320 S. (65) Loewig, C., et A. Kölliker: Observations sur l'éxistence d'une substance ternaire identique avec la cellulose dans les Tuniciers. C. r. Acad. Sci. Paris 22, 38 (1846). (66) Lohmann, H.: Die Appendicularien der Plankton-Expedition. Erg. Plankton. Exp. 2 (1896). (67) (1) Das Gehäuse der Appendicularien, sein Bau, seine Funktion und seine Entstehung. Schr. naturwiss. Ver. Schlesw.-Holst. 11, 345 (1899). (68) (2) Das Gehäuse der Appendicularien nach seiner Bildungsweise, seinem Bau und seiner Funktion. Zool. Anz. 22, 206 (1899). (69) Die Appendicularien. Fauna arct. 1, 363 (1900). (70) Die Appendicularien. Nord. Plankton 1, 11 (1901). (71) Gehäuse und Gallertblasen der Appendicularien und ihre Bedeutung für die Erforschung des Lebens im Meere. Verh. dtsch. zool. Ges. 19, 200 (1909). (72) Die von Sekretfäden gebildeten Fangapparate im Tierreich und ihre Erbauer. Mitt. naturhist. Mus. Hamburg 30, 255 (1912). (73) Tunicata. In Handwörterbuch der Naturwissenschaften, Bd. 10, S. 57—90. Jena 1913. (74) Die Appendicularien der Valdivia-Expedition. Verh. dtsch. zool. Ges. 24, 157 (1914). (75) Die Appendicularien der Deutschen Tiefsee-Expedition. Erg. dtsch. Tiefs.-Exp. 21, 1 (1931). (76) Tunicata — Appendiculariae. In Handbuch der Zoologie, Bd. 5, S. 3—202. Berlin 1933. (77) Lohmann, H. u. A. Bückmann: Die Appendicularien der Deutschen Südpolar-Expedition. Dtsch. Südp.-Exp. 18, 63 (1926). (78) Martini, E.: (1) Studien über die Konstanz histologischer Elemente. I. Z. Zool. 92, 563 (1909). (79) (2) Über Eutelie und Neotenie. Verh. dtsch. zool. Ges. 19, 292 (1909). (80) Studien über die Konstanz histologischer Elemente. II. Z. Zool. 94, 81 (1910). (81) Meek, A.: The transmutation of *Oikopleura dioica*. Rep. Mar. Lab. Cullercoats N. S. 18, 47 (1929). (82) Meves, F.: Zur Struktur der Kerne in den Spinndrüsen der Raupen. Arch. mikrosk. Anat. 48, 573 (1897). (83) Moulin, F. W. K. de: Struktur des Zellprotoplasmas. Z. Zellforschg 1, 307 (1924). (84) Neumann, G.: (1

Thaliacea — Pyrosomida. In Handbuch der Zoologie, Bd. 5, S. 203—323. Berlin 1935. (2) Cyclomyaria. In Handbuch der Zoologie, Bd. 5, S. 324. Berlin 1935. (89) Parat, M., et L. Painlevé: Cellule glandulaire en activité. C. r. Acad. Sci. Paris 179, 612 (1924). (90) Payen, L.: Zit. nach Huus. Handbuch der Zoologie, Bd. 5, S. 557. 1937. (91) Prenant, M.: Prochordes, Tuniciers. Actual. Sci. industr., 1, 380 (1936). (92) Ries, E.: (1) Die Tropfzellen und ihre Bedeutung für die Tunicabildung bei Clavellina. Arch. Entw.mechan. 137, 363 (1937). (93) (2) Lebenszyklen und Arbeitsrhythmus von Zellen. Verh. dtsch. zool. Ges. 39, 171 (1937). (94) Risler, H.: Kernvolumenänderungen in der Raupenepidermis von *Ptychopoda seriata*. Z. Naturforschg 3b, 129 (1948). (95) Roch, A.: Tunicata. Tabulae biol. 6, 536 (1930). (96) Romeis, B.: Mikroskopische Technik, 15. Aufl. München: Leibniz 1948. 695 S. (97) Saint-Hilaire, K.: Morphogenetische Untersuchungen des Ascidienmantels. Zool. Jb., Anat. 54, 435 (1931). (98) Salensky, W.: Études anatomiques sur les Appendiculaires. I. Mém. Acad. Sci. St. Pétèrsbourg 13, 1 (1913). (99) Études anatomiques sur les Appendiculaires. II. Mém. Acad. Sci. St. Pétèrsbourg 15, 135 (1904). (100) Schäfer, W.: 1871 zit. nach Neumann, Handbuch der Zoologie, Bd. 5, S. 233. 1935. (101) Schmidt, C.: Über Kernveränderungen in den Sekretionszellen. Diss. Breslau 1882. (102) Schulze, F. E.: Über die Struktur des Tunikatenmantels und sein Verhalten im polarisierten Licht. Z. Zool. 12, 175 (1863). (103) Schulze, P., u. G. Kunike: Zur Mikrochemie tierischer Skelettsubstanzen. Biol. Zbl. 43, 556 (1923). (104) Seeliger, O.: (1) Einige Beobachtungen über die Bildung des äußeren Mantels der Tunikaten. Z. Zool. 56, 488 (1893). (105) (2) Tunicata. In Bronns Klassen und Ordnungen des Tierreichs, Bd. 3, S. 1—1773. 1893. (106) Semper, C.: Über die Entstehung der geschichteten Zellulosepidermis der Ascidien. Arb. zool. Inst. Würzburg 2 (1875). (107) Sutra, R.: Études sur les celluloses d'Acetobacter xylinum et des Tuniciers. C. r. Acad. Sci. Paris 195, 181 (1932). (108) Thompson, H.: Pelagic Tunicates of Australia. Commonw. Sci. Industr. Res., Melbourne 1948, 196 S. (109) Uljanin, E.: Über die embryonale Entwicklung von Doliolum. Zool. Anz. 3, 473 (1881). (110) Zur Naturgeschichte von Doliolum. Zool. Anz. 5, 429 (1882). (111) Vernieres, P.: Les Appendiculaires de la mer de Villefranche. Bull. Soc. zool. France 59, 160 (1934). (112) Voinov, D. N.: Mécanismes secretoires des cellules. C. r. Soc. Biol. Paris 101, 323 (1928). (113) Wermel, E., u. W. W. Portulagow: Über den Nachweis des rhythmischen Zellwachstums. Z. Zellforschg 22 (1935). (114) Zeiger, K.: Zum Problem der vitalen Struktur des Zellkernes. Z. Zellforschg 22 (1935).

Translation of German and Dutch bibliographical entries

2. Cellulose shell in arthopods and molluscs.

3. Pleochroism of stained anisotropic substances in the animal body.

4. On the glandular structure and secretion of the frog skin.

5. Microscopic-chemical analysis of glycogen and some other polysaccharides. p. 51

6. On some matters of dispute regarding cell division.

7. On the application of the ninhydrin reaction in microscopic preparations to identify low protein substances.

10. Notes on appendicularians from the collection of the German Antarctic Expedition.

11. Contribution to the knowledge of appendicularians from the collection of the German Antarctic Expedition.

12. Copelata. Fauna of the North Sea and the Baltic Sea.

13. Tunicata. Fauna of Germany.

14. Appendicularia I - III.

15. On the chemical composition of the nuclear structures.

17. Additional observations on the development of Oikopleura dioica.

18. The origin of vertebrates.

23. The submicroscopic structure of the cell membranes.

24. The nature of the zinc chloroiodide reaction.

25. Submicroscopic morphology of the protoplasm and its derivatives.

26. Tunicin in the electron microscope.

31. Fundamentals of cytology.

32. Growth of the nucleus in animal and plant tissues.

33. Studies on the role of the nucleoli in the nucleus.

35. On the enzymatic decomposition of cellulose and its products of decomposition.

36. Textbook of zoology.

37. Experiments involving microchemical reactions.

40. Studies of the structure and the development of the cellulose tunic in tunicates.

42. The problem of restitution of glandular secretion.

43. Cytoplasmic structures in tunicates, sponges and protozoan cells. p. 52

44. Studies of the distribution and role of chromotropic substances.

45. Ascidiaceae. In: Handbook of zoology.

46. Contribution to the knowledge of the morphology and systematics of the appendicularians.

47. On the endostyle and the systematic position of the appendicularians.

48. The appendicularians of the Siboga Expedition.

49. On the so-called metameric segmentation of the appendicularian tail.

50. The appendicularians.

51. Desmomyaria. In: Handbook of zoology.

53. Textbook of organic chemistry.

54. On nuclear changes in the ectoderm of the appendicularians during formation of the house.

55. Contributions to the morphology and physiology of the nucleus.

56. Comparative animal evolution. Tunicates.

57. Some contributions to the formation of the tunic in the ascidians.

58. The role of the nucleus in cellular processes.

59. The behaviour of the hypodermis and of the Versonian glands in response to the pupation hormone in Ephestia kühniella.

60. Identification and distribution of organic skeletal substances in animals.

62. Chemical physiology.

63. Zoological studies. Appendicularia albicans.

66. The appendicularians of the Plankton Expedition.

67. The house of the appendicularians, its structure, function and development.

68. The house of the appendicularians with its formation, its structure and its function.

69. The appendicularians.

70. The appendicularians.

71. House and gelatinous vesicles of the appendicularians and their importance for the study of life in the sea.

72. Feeding apparatuses composed of secretory filaments and their builders in the animal kingdom.

73. Tunicata. In: Dictionary of natural sciences.

74. The appendicularians of the Valdivia Expedition.

75. The appendicularians of the German Deep Sea Expedition.

76. Appendiculariae. In: Handbook of zoology.

77. The appendicularians of the German Antarctic Expedition.

78. Studies of the constancy of histological elements. Part I.

79. On eutely and neoteny.

80. Studies of the constancy of histological elements. Part II.

82. On the structure of the nuclei in the spinning glands of the caterpillars.

83. Structure of protoplasm.

84. Thaliaceae - Pyrosomida. In: Handbook of zoology. p. 53

85. Cyclomyaria. In: Handbook of zoology.

87. Quotation from HUUS. Handbook of zoology.

89. Secretory cells and their role in the formaton of the tunic in Clavellina.

90. Life cycles and activity rhythm of cells.

91. Changes in the size of the nuclei in the epidermis of the caterpillar in Plychopoda seriata.

93. Micro-techniques.

94. Morphogenetic studies of the ascidian tunic.

97. 1871, quoted from NEUMANN. Handbook of zoology.

98. Nuclear changes in secretory cells.

99. On the structure of the tunic in tunicates and its behaviour in polarized light.

100. On the micro-chemistry of skeletal substances in animals.

101. Some observations on the formation of the outer tunic in tunicates.

102. Tunicata. In: BRONN's classes and orders of the animal kingdom.

103. On the development of the stratified cellulose epidermis in the ascidians.

106. On the embryonic development of Doliolum.

107. On the natural history of Doliolum.

110. Evidence of rhythmic growth of cells.

111. The problem of the vital structure of the nucleus.

Author's postal address:

Dr. W.F. Körner
Zoolog. Institut der Techn. Hochschule (Zoological Department of
Darmstadt the Institute of Technology)
Federal Republic of Germany